533 Rec'd PCT/PTO 18 SEP 2001

JC152 PATENT & TRADEMARK OFFICE SEP 18 2001

FORM PTO-1390 (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		ATTORNEY'S DOCKET NO. 05407.00003 U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 09/936845
INTERNATIONAL APPLICATION NO. PCT/GB00/01035	INTERNATIONAL FILING DATE March 20, 2000	PRIORITY DATE CLAIMED March 18, 1999 and Feb. 18, 2000
TITLE OF INVENTION POLYSATURATED FATTY ACID (PUFA) ELONGASE FROM CAENORHABDITIS ELEGANS		
APPLICANT(S) FOR DO/EO/US Johnathan A. NAPIER		

Applicant herewith submits to the United State Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371 (c)(2).
   a ☐ is attached hereto.
   b ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11-20 below concern other document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.
12. ☒ An Assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☒ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821-1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4)..
20. ☒ Other items or information: PCT/RO/101 (4 pp.); PCT/IPEA/401 (3 pp.); PCT/IPEA/409 (5 pp.); PCT/ISA/210 (9 pp.); Copy of WO 00/55330 published September 21, 2000 w/PCT/ISA/210: Specification (27 pp.), Claims 1-40 (3 pp.), 4 sheets of Drawings, Abstract

U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 09/936845	INTERNATIONAL APPLICATION NO. PCT/GB00/01035	ATTORNEY'S DOCKET NO. 05407.00003

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO ..... $1,000.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO..... $ 860.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.455(a)(2)) paid to USPTO ..... $ 710.00 International preliminary examination fee (37 CFR 1,482) paid to USPTO) but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... $ 690.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) ..... $ 100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$860.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	43 -20 =	23	X $18.00	$414.00	
Independent Claims	1 - 3 =	0	X $ 80.00	$	
Multiple dependent claims (if applicable)			X $270.00	$	
TOTAL OF ABOVE CALCULATIONS =				$1,274.00	
☐Applicant claims small entity status. See 37 CFR 1.27. The fees indicated below above are reduced by 1/2.				$	
SUBTOTAL =				$1,274.00	
Processing fee of $130.00 for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f).				$	
TOTAL NATIONAL FEE =				$1,274.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property.				$ 40.00	
TOTAL FEES ENCLOSED =				$1,314.00	
+				Amount to be: refunded	$
				charged	$

a. ☐ A check in the amount of $_____ to cover the above fees is enclosed.
b. ☒ Please charge my Deposit Account No. 19-0733 in the amount of $1,314.00 to cover the above fees. A duplicate copy of this sheet is enclosed.
c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 19-0733. A duplicate copy of this sheet is enclosed.
d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Banner & Witcoff, Ltd.
Eleventh Floor
1001 G Street, N.W.
Washington, D.C. 20001-4597
Telephone (202) 508-9100
Date: September 18, 2001

Lisa M. Hemmendinger
SIGNATURE

Lisa M. Hemmendinger
NAME

42,653
REGISTRATION NUMBER

09/936845
JC16 Rec'd PCT/PTO SEP 18 2001

Attorney Docket No. 05407.00003
International Application No. PCT/GB00/01035

PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Johnathan A. NAPIER .

BOX PCT

National Phase Application
PCT/GB00/01035
Filed: March 20, 2000

Serial No.: Unassigned

Group Art Unit: Unassigned

Filed: CONCURRENTLY HEREWITH

Examiner: Unassigned

For: POLYSATURATED FATTY ACID (PUFA) ELONGASE FROM CAENORHABDITIS ELEGANS

LETTER PURSUANT TO 37 CFR 1.821(f)

Assistant Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

In the matter of the above-identified application, which is filed concurrently herewith, Applicants submit a computer diskette containing the sequences of the instant application. It is hereby certified that the paper and computer copies of these sequences are identical in content.

Respectfully submitted,

Lisa M. Hemmendinger
Reg. No. 42,653

September 18, 2001
BANNER & WITCOFF, LTD.
Eleventh Floor
1001 G Street, N.W.
Washington, D.C. 20001-4597
(202) 508-9100

PTO/PCT Rec'd 06 MAR 2002

#3.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)	
	)	Group Art Unit: TBA
NAPIER	)	
	)	Examiner: TBA
Serial No. 09/936,845	)	
	)	
Filed: September 28, 2001	)	Atty. Dkt. No. 05407.00003

For: **POLYUNSATURATED FATTY ACID (PUFA) ELONGASE FROM CAENORHABDITIS ELEGANS (AS AMENDED)**

AMENDMENT

Assistant Director for Patents
Washington, D.C. 20231

Sir:

This amendment is filed in response to the Notification of Missing Requirements mailed January 8, 2002. Accompanying this amendment are:

- a paper copy of a substitute sequence listing;
- a computer readable form of the substitute sequence listing; and
- a copy of the Notification of Missing Requirements.

We believe no fee is due in connection with this amendment. If a fee is due, please charge our Deposit Account No. 19-0733.

Please enter the following amendment.

IN THE SPECIFICATION

(1) Delete the sequence listing insert the paper copy of the substitute sequence listing at the

end of the application.

Remarks

Paper and computer readable forms of a substitute sequence listing accompany this paper. The contents of the substitute sequence listing are identical to the sequence listing originally filed except for the identification of amino acid "variants" in SEQ ID NO:22. The substitute sequence listing adds no new matter to the application.

I believe the contents of the computer readable form and the paper copy of the substitute sequence listing are identical.

Respectfully submitted,

Date: March 6, 2002

By: Lisa M. Hemmendinger
Lisa M. Hemmendinger
Registration No. 42,653

SCANNED, #12

Banner & Witcoff, Ltd.
1001 G Street, N.W., Eleventh Floor
Washington, D.C. 20001-4597
(202) 508-9100

09/936845
JC16 Rec'd PCT/PTO SEP 18 2001

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)	
	)	Group Art Unit:
NAPIER	)	
	)	Examiner:
Serial No. TBA	)	
	)	
Filed: September 28, 2001	)	Atty. Dkt. No. 05407.00003

For: POLYUNSATURATED FATTY ACID (PUFA) ELONGASE FROM CAENORHABDITIS ELEGANS (AS AMENDED)

PRELIMINARY AMENDMENT

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Please enter the following amendments before examining the application referenced above. We believe no fee is due in connection with this filing. If a fee is due, please charge Deposit Account No. 19-0733.

Appendix 1 is a copy of the amended paragraphs, with markings to show changes made. Appendix 2 is a copy of the amended claims, with markings to show changes made.

IN THE SPECIFICATION

(1) On page 1, delete the title and substitute therefore:

POLYUNSATURATED FATTY ACID (PUFA) ELONGASE FROM CAENORHABDITIS ELEGANS

(2) On page 1, after the title, insert the following paragraph:

This application claims the benefit of and incorporates by reference the following applications: PCT/GB00/01035 filed March 20, 2000, 9906307.5 filed March 18, 1999, and 0003869.5 filed

February 19, 2000, all of which were published in English.

(3) On page 3, delete the first full paragraph and substitute the following paragraph:

In order to identify genes encoding PUFA elongases, it is necessary to study systems in which the synthesis of PUFAs is well documented; a good example of this is the model animal system *C. elegans*, a small free-living worm (Tanaka *et al.*, (1996), *Lipids* 31, 1173-78). *C. elegans*, like most other animals, and in contrast to higher plants, synthesizes PUFAs such as arachidonic acid (AA; 20:4 $\Delta^{5, 8, 11, 14}$) as precursors to a class of molecules known as the eicosanoids, which in turn serve as precursors for compounds such as prostaglandins and leucotriens (Horrobin, (1990), *Review in Contemp Pharmacotherapy*, 1:1-45). The presence of AA and other long chain polyunsaturated fatty acids in *C. elegans* is well documented (Tanaka *et al.*, (1996), *Lipids* 1, 1173-1178). The complete sequence of the nematode's genome is now publicly available (*The C. elegans cosortium, 1998, Science 282, 2012-2018.* See the database at the website identified with the URL file type, www host server, domain name sanger.ac.uk and following the path from "Projects" to "C_elegans" to "blast_server.shtml".

(4) On page 7, delete the paragraph beginning on line 18 and extending to page 8, line 21 and replace it with the following paragraph:

Initially the *C. elegans* databases were searched for any sequences which showed low levels of homology to yeast ELO genes (*ELO2* and *ELO3*) using the TBLASTN programme. A similar search was carried out using short (20 to 50 amino acid) stretches of ELO genes which were conserved amongst the three ELO polypeptide sequences. *C. elegans* sequences which were identified by this method were then used themselves as search probes, to identify any related *C. elegans* genes which the initial search with the yeast sequences failed to identify. This was necessary because the level of homology between the yeast ELO genes and any worm genes is always low (see BLAST scores later). To allow for a more sensitive search of worm sequences, a novel approach was adopted to circumvent the major drawback with searches using the BLAST programmes, namely that the search string (i.e. the input search motif) must be longer than 15 characters for the algorithm to work. Thus, if it was desired to search for a short motif (like a

histidine box), then the BLAST programme would not be capable of doing this. A complete list of all the predicted ORFs present in the *C. elegans* genome exists as a database called Wormpep, which is freely available from the Sanger WWW site identified with the URL address http file type, www host server, domain name sanger.ac.uk and following the path from "Projects" to "C_elegans" to "webace_front_end.shtml". The latest version of Wormpep was down loaded to the hard disc of a Pentium PC, and re-formatted as a Microsoft Word6 document, resulting in a document of about 3,500 pages. This was then searched using the "Search & Replace" function of Word6, which also allows for the introduction of "wildcard" characters into the search motif. So, for example, it is possible to search both for the short text string HPGG, which would identify any predicted worm ORF present in the Wormpep 3,500 page document containing this motif, or alternatively search with HPGX (where X is a wild card character). Clearly, such (manual) searches of a 3,500 page document are extremely time-consuming and demanding, also requiring visual inspection of each and every identified ORF. For example, searching with a motif such as HXXHH identifies in excess of 300 different ORFs. However, by using a number of different short search strings (as outlined below), and combining these with other methods for identifying putative elongase enzymes, a number of candidate ORFs have been identified.

(5) On page 8, delete the paragraph beginning at line 23 to and extending to page 9, line 3 and substitute the following paragraph:

As a negative control, to demonstrate that the FAE1 gene sequence was unlikely to provide a useful search sequence in the identification of *C. elegans* sequences encoding for PUFA elongases, the GenBank databases identified with the URL address http file type, www host server, domain name "ncbi.nlm.nih.gov" and following the path from Web to Search to index.html were searched using the *Arabidopsis* FAE1 polypeptide sequence ot identify related genes or expressed sequence transcripts (ESTs). GenBank is the NIH genetic sequence database, an annotated collection of all publicly available DNA sequences (*Nucleic Acid Research* (1998) 26, 1-7). There are approximately 2,162,000,000 bases in 3,044,000 sequence records as of December 1998. The search was carried out using the BLAST2 (Basic Local Alignment Search Tool) algorithm (Altschul *et al.*, (1990) *J Mol Biol* 215, 403, 410). Although a number of plant

ORFs and ESTs were reported as being related, o animal sequences were identified by this search, confirming the observation that FAE1 was unlikely to be a suitable candidate as a search template for PUFA elongases.

(6) On page 9, delete the paragraph beginning on line 5 and substitute the following paragraph:

Using the three yeast fatty acid elongase sequences (ELO 1, 2, 3) as probes, a number of putative ORFs in the DNA of *C. elegans*-derived cosmid sequences which form the *C. elegans* genomic sequence database was identified. Moreover, an extensive and time-consuming search of a downloaded copy of the WormPep database identified with the URL address ftp file type, ftp host server, domain name sanger.ac.uk, following the path from "pub" to "databases" to "wormpep" using manual search strings in MSWord 6, identified a number of *C. elegans* ORFs which contained presumptive histidine boxes. Wormpep contains predicted proteins from the *Caenorhabditis elgans* genome sequence project, which is carried out jointly by the Sanger Centre in Cambridge, UK and Genome Sequencing Center in St. Louis, USA. The current Wormpep database, Wormpep 16, contains 16,332 protein sequences (7,120,115 residues). Search strings used included [HXXHH], [HXXXHH], [QXXHH] and [YHH]. Comparison of the data from the two different searches indicated a small (<10) number of putative ORFs as candidate elongases. The histidine box motifs are located at amino acids 162-166 of SEQ ID NO:15, amino acids 186-190 of SEQ ID NO:16, amino acids 145-150 of SEQ ID NO:17, amino acids 147-151 of SEQ ID NO:18, amino acids 141-145 of SEQ ID NO:19, amino acids 177-181 of SEQ ID NO:20, amino acids 155-159 of SEQ ID NO:21, and amino acids 233-237 of SEQ ID NO:22.

(7) On page 10, delete the paragraph beginning on line 7 and substitute the following paragraph:

Since the inventors had previously observed that *C. elegans* genes involved in the synthesis of PUFA may exist in tandem (for example the Δ5 and Δ6 desaturases required for AA and GLA synthesis, respectively, are <1 kB apart on chromosome IV (Michaelson et al., (1998), *FEBS Letts* **439**, 215-218), the positions of the putative *C. elegans* elongase ORFs were determined

using the Sanger Centre's WebAce *C. elegans* server identified with the URL address http filetype, www host server, domain name sanger.ac.uk and following the path from "Projects" to C_elegans" to "webac_front_ends.shtml". This indicated that two pairs of putative elongases were in close proximity to each other on the *C. elegans* chromosome IV.

(8) On page 13, delete the paragraph beginning at line 2 and substitute the following paragraph:

Putative elongase sequences F56H11.4 and F41H10.8 were cloned by PCR into the pYES2 vector (Invitrogen). A *C. elegans* mixed stage cDNA library was used as a PCR template.
F56H11.4 was amplified using primers:
56h114.for 5'-GCGGGTACCATGGCTCAGCATCCGCTC-3' (SEQ ID NO:1) and;
56h114.rev 5'-GCGGGATCCTTAGTTGTTCTTCTTCTT-3' (SEQ ID NO:2).
F41H10.8 was amplified using primers:
41h108.for 5'-GCGGGTACCATGCCACAGGGAGAAGTC-3' (SEQ ID NO:3) and;
416h108.rev 5'-GCGGGATCCTTATTCAATTTTTCTTTT-3' (SEQ ID NO:4).

(9) On page 13, delete the paragraph beginning on line 13 and substitute the following paragraph:

An ORF encoding the *Mortierella alpina* Δ^5-fatty acid desaturase (Michaelson, L.V., et al. (1998) *J. Biol. Chem.*, **273**, 19055-19059) was amplified using primers:
Mad5.for 5'-GCGAATTCACCATGGGTACGGACCAAGGA-3' (SEQ ID NO:5) and;

Mad5.rev 5'-GCGGAGCTCCTACTCTTCCTTGGGACG-3' (SEQ ID NO:6).

(10) Delete the informal sequence listing at pages 22-27 and insert the paper copy of the formal sequence listing at the end of the application.

IN THE CLAIMS

1. (Amended) An isolated polypeptide comprising a functional long chain polyunsaturated fatty acid (PUFA) elongase having a function of extending a chain length of an 18 carbon PUFA to 20 carbons in length.

3. (Amended) A polypeptide according to claim 1 wherein the polypeptide comprises a portion of the amino acid sequence shown in SEQ ID NO:15 or a variant thereof.

7. (Amended) A polypeptide according to claim 1 wherein the polypeptide sequence includes a sequence motif responsible for Endoplasmic Reticulum (ER)-retention.

8. (Amended) A polypeptide according to claim 1 wherein the polypeptide is capable of elongating palmitoleic acid (PA; $16:1\Delta^{9}$) to vacceric acid (VA; $18:1\Delta^{11}$).

9. (Amended) A polypeptide according to claim 1 wherein the polypeptide is an animal polypeptide.

16. (Amended) An isolated DNA molecule encoding a polypeptide according to claim 1.

17. (Amended) A DNA molecule according to claim 16 wherein the DNA molecule comprises the sequence shown in SEQ ID NO:7 or variants of that sequence due to base substitutions, deletions, and/or additions.

18. (Amended) An engineered organism engineered to express a polypeptide according to claim 1.

21. (Amended) An engineered organism containing a synthetic pathway for the production of a polypeptide according to claim 1.

23. (Amended) An engineered organism according to claim 21 wherein the pathway includes Δ^{6}-fatty acid desaturase.

24. (Amended) An engineered organism according to claim 21 wherein the organism is a lower eukaryote.

27. (Amended) A transgenic plant engineered to express a polypeptide according to claim1.

28. (Amended) A transgenic plant containing a DNA molecule according to claim 16.

29. (Amended) A method of producing a PUFA comprising carrying out an elongase reaction catalysed by a polypeptide according to claim 1.

32. (Amended) A PUFA produced by a method according to claim 29.

35. (Amended) A pharmaceutical composition comprising a polypeptide according to claim 1.

37. (Amended) A pharmaceutical composition according to claim 35 wherein the composition comprises a pharmaceutically-acceptable diluent, carrier, excipient or extender.

38. (Amended) A method of elevating the PUFA levels of an animal or a plant comprising the step of supplying to the animal or plant a polypeptide according to claim 1.

39. (Amended) A method according to claim 38 wherein the animal is a mammal.

40. (Amended) A method according to claim 39 wherein the mammal is a human.

41. (New) A pharmaceutical composition according to claim 36 wherein the composition comprises a pharmaceutically-acceptable diluent, carrier, excipient, or extender.

42. (New) A method of elevating the PUFA levels of an animal or a plant comprising the step of supplying the animal or plant with a DNA molecule according to claim 16.

43. (New) A method of elevating the PUFA levels of an animal or a plant by supplying the animal or plant with a PUFA according to claim 32.

Remarks

Amendments to the Claims and Specification

The claims are amended to remove multiple dependencies. New claim 41-43 is supported by original claim 37. New claims 42 and 43 are supported by original claim 38.

The specification is amended to delete hyperlinks and to insert sequence identifiers. The amendments add no new matter to the specification.

Formal Sequence Listing

A paper and a computer readable form of a formal sequence listing accompany this amendment. I believe the sequence contents of the paper and computer readable forms are identical.

The formal sequence listing adds no new matter to the specification. It contains the primer sequences disclosed at page 13 as SEQ ID NOS:1-6. SEQ ID NOS:7-22 are the sequences present in the informal sequence listing as SEQ ID NOS:1-16, respectively.

Respectfully submitted,

Date: September 18, 2001

By: Lisa M. Hemmendinger

Lisa M. Hemmendinger
Registration No. 42,653

Banner & Witcoff, Ltd.
1001 G Street, N.W., Eleventh Floor
Washington, D.C. 20001-4597
(202) 508-9100

Appendix 1. Version of Amended Paragraphs with Markings to Show Changes Made

On page 1, in the title:

[POLYSATURATED] POLYUNSATURATED FATTY ACID (PUFA) ELONGASE FROM CAENORHABDITIS ELEGANS

Page 3, first full paragraph:

In order to identify genes encoding PUFA elongases, it is necessary to study systems in which the synthesis of PUFAs is well documented; a good example of this is the model animal system *C. elegans*, a small free-living worm (Tanaka *et al.*, (1996), *Lipids* 31, 1173-78). *C. elegans*, like most other animals, and in contrast to higher plants, synthesizes PUFAs such as arachidonic acid (AA; 20:4 $\Delta^{5, 8, 11, 14}$) as precursors to a class of molecules known as the eicosanoids, which in turn serve as precursors for compounds such as prostaglandins and leucotriens (Horrobin, (1990), *Review in Contemp* [*Pharmacotherpy*] *Pharmacotherapy*, 1:1-45). The presence of AA and other long chain polyunsaturated fatty acids in *C. elegans* is well documented (Tanaka *et al.*, (1996), *Lipids* 1, 1173-1178). The complete sequence of the nematode's genome is now publicly available (*The C. elegans cosortium, 1998, Science 282, 2012-2018.* See the database at the website identified with the URL file type, www host server, domain name sanger.ac.uk and following the path from "Projects" to "C_elegans" to "blast_server.shtml" [*Database at http://www.sanger.ac.uk/Projects/C_elegans/blast_server.shtml)*].

Page 7, line 18 to page 8, line 21:

Initially the *C. elegans* databases were searched for any sequences which showed low levels of homology to yeast ELO genes (*ELO2* and *ELO3*) using the TBLASTN programme. A similar search was carried out using short (20 to 50 amino acid) stretches of ELO genes which were conserved amongst the three

ELO polypeptide sequences. *C. elegans* sequences which were identified by this method were then used themselves as search probes, to identify any related *C. elegans* genes which the initial search with the yeast sequences failed to identify. This was necessary because the level of homology between the yeast ELO genes and any worm genes is always low (see BLAST scores later). To allow for a more sensitive search of worm sequences, a novel approach was adopted to circumvent the major drawback with searches using the BLAST programmes, namely that the search string (i.e. the input search motif) must be longer than 15 characters for the algorithm to work. Thus, if it was desired to search for a short motif (like a histidine box), then the BLAST programme would not be capable of doing this. A complete list of all the predicted ORFs present in the *C. elegans* genome exists as a database called Wormpep, which is freely available from the Sanger WWW site identified with the URL address http file type, www host server, domain name sanger.ac.uk and following the path from "Projects" to "C_elegans" to "webace_front_end.shtml" [(http://www.sanger.ac.uk/Projects/C_elegans/webace_front_end.shtml)]. The latest version of Wormpep was down loaded to the hard disc of a Pentium PC, and re-formatted as a Microsoft Word6 document, resulting in a document of about 3,500 pages. This was then searched using the "Search & Replace" function of Word6, which also allows for the introduction of "wildcard" characters into the search motif. So, for example, it is possible to search both for the short text string HPGG, which would identify any predicted worm ORF present in the Wormpep 3,500 page document containing this motif, or alternatively search with HPGX (where X is a wild card character). Clearly, such (manual) searches of a 3,500 page document are extremely time-consuming and demanding, also requiring visual inspection of each and every identified ORF. For example, searching with a motif such as HXXHH identifies in excess of 300 different ORFs. However, by using a number of different short search strings (as outlined below), and combining these with other methods for identifying putative elongase enzymes, a number of candidate ORFs have been identified.

Page 8, line 23 to page 9, line 3:

As a negative control, to demonstrate that the FAE1 gene sequence was unlikely to provide a useful search sequence in the identification of *C. elegans* sequences encoding for PUFA elongases, the GenBank databases [(http://www.ncbi.nlm.nih.gov/Web/Search/index.html)] identified with the URL address http file type, www host server, domain name "ncbi.nlm.nih.gov" and following the path from Web to Search to index.html were searched using the *Arabidopsis* FAE1 polypeptide sequence ot identify related genes or expressed sequence transcripts (ESTs). GenBank is the NIH genetic sequence database, an annotated collection of all publicly available DNA sequences (*Nucleic Acid Research* (1998) 26, 1-7). There are approximately 2,162,000,000 bases in 3,044,000 sequence records as of December 1998. The search was carried out using the BLAST2 (Basic Local Alignment Search Tool) algorithm (Altschul *et al.*, (1990) *J Mol Biol* 215, 403, 410). Although a number of plant ORFs and ESTs were reported as being related, o animal sequences were identified by this search, confirming the observation that FAE1 was unlikely to be a suitable candidate as a search template for PUFA elongases.

Page 9, line 5:

Using the three yeast fatty acid elongase sequences (ELO 1, 2, 3) as probes, a number of putative ORFs in the DNA of *C. elegans*-derived cosmid sequences which form the *C. elegans* genomic sequence database was identified. Moreover, an extensive and time-consuming search of a downloaded copy of the WormPep database identified with the URL address ftp file type, ftp host server, domain name sanger.ac.uk, following the path from "pub" to "databases" to "wormpep" [(ftp://ftp.sanger.ac.uk/pub/databases/wormpep)] using manual search strings in MSWord 6, identified a number of *C. elegans* ORFs which contained presumptive histidine boxes. Wormpep contains predicted proteins from the *Caenorhabditis elgans* genome sequence project, which is

carried out jointly by the Sanger Centre in Cambridge, UK and Genome Sequencing Center in St. Louis, USA. The current Wormpep database, Wormpep 16, contains 16,332 protein sequences (7,120,115 residues). Search strings used included [HXXHH], [HXXXHH], [QXXHH] and [YHH]. Comparison of the data from the two different searches indicated a small (<10) number of putative ORFs as candidate elongases. The histidine box motifs are [shown in bold in SEQ ID 9 to 16] located at amino acids 162-166 of SEQ ID NO:15, amino acids 186-190 of SEQ ID NO:16, amino acids 145-150 of SEQ ID NO:17, amino acids 147-151 of SEQ ID NO:18, amino acids 141-145 of SEQ ID NO:19, amino acids 177-181 of SEQ ID NO:20, amino acids 155-159 of SEQ ID NO:21, and amino acids 233-237 of SEQ ID NO:22.

Page 10, line 7:

Since the inventors had previously observed that *C. elegans* genes involved in the synthesis of PUFA may exist in tandem (for example the Δ5 and Δ6 desaturases required for AA and GLA synthesis, respectively, are <1 kB apart on chromosome IV (Michaelson et al., (1998), *FEBS Letts* **439**, 215-218), the positions of the putative *C. elegans* elongase ORFs were determined using the Sanger Centre's WebAce *C. elegans* server identified with the URL address http filetype, www host server, domain name sanger.ac.uk and following the path from "Projects" to C_elegans" to "webac_front_ends.shtml" [(http://www.sanger.ac.uk/Projects/C_elegans/webac_front_ends.shtml).]. This indicated that two pairs of putative elongases were in close proximity to each other on the *C. elegans* chromosome IV.

Page 13, line 2:

Putative elongase sequences F56H11.4 and F41H10.8 were cloned by PCR into the pYES2 vector (Invitrogen). A *C. elegans* mixed stage cDNA library was used as a PCR template. F56H11.4 was amplified using primers:

56h114.for 5'-GCGGGTACCATGGCTCAGCATCCGCTC-3' (SEQ ID NO:1) and;

56h114.rev 5'-GCGGGATCCTTAGTTGTTCTTCTTCTT-3' (SEQ ID NO:2).

F41H10.8 was amplified using primers:

41h108.for 5'-GCGGGTACCATGCCACAGGGAGAAGTC-3' (SEQ ID NO:3) and;

416h108.rev 5'-GCGGGATCCTTATTCAATTTTTCTTTT-3' (SEQ ID NO:4).

Page 13, line 13:

An ORF encoding the *Mortierella alpina* Δ^5-fatty acid desaturase (Michaelson, L.V., et al. (1998) *J. Biol. Chem.*, **273**, 19055-19059) was amplified using primers:

Mad5.for 5'-GCGAATTCACCATGGGTACGGACCAAGGA-3' (SEQ ID NO:5) and;

Mad5.rev 5'-GCGGAGCTCCTACTCTTCCTTGGGACG-3' (SEQ ID NO:6).

Appendix 2. Version of Amended Claims with Markings to Show Changes Made

1. (Amended) An isolated polypeptide comprising a functional long chain polyunsaturated fatty acid (PUFA) elongase [as herein defined] having a function of extending a chain length of an 18 carbon PUFA to 20 carbons in length.

3. (Amended) A polypeptide according to claim 1 [or claim 2] wherein the polypeptide [has at least] comprises a portion of the amino acid sequence shown in SEQ ID NO:15 or a variant [variants] thereof.

7. (Amended) A polypeptide according to [any preceding] claim 1 wherein the polypeptide sequence includes a sequence motif responsible for Endoplasmic Reticulum (ER)-retention.

8. (Amended) A polypeptide according to [any preceding] claim 1 wherein the polypeptide is capable of elongating palmitoleic acid (PA; $16:1\Delta^{9}$) to vacceric acid (VA; $18:1\Delta^{11}$).

9. (Amended) A polypeptide according to [any preceding] claim 1 wherein the polypeptide is [from] an animal polypeptide.

16. (Amended) [A] An isolated DNA [sequence] molecule encoding a polypeptide according to [any preceding] claim 1.

17. (Amended) [A] A DNA [sequence] molecule according to claim 16 wherein the DNA molecule comprises the sequence shown in SEQ ID NO:7 or variants of that sequence due to base substitutions, deletions, and/or additions.

18. (Amended) An engineered organism engineered to express a polypeptide according to [any one of claims] claim 1.

21. (Amended) An engineered organism containing a synthetic pathway for the production of a polypeptide according to [any one of claims] claim 1.

23. (Amended) An engineered organism according to claim 21 [or 22] wherein the pathway includes Δ^6-fatty acid desaturase.

24. (Amended) An engineered organism according to [any one of claims] claim 21 [to 23] wherein the [animal] organism is a lower eukaryote.

27. (Amended) A transgenic plant engineered to express a polypeptide according to [any one of claims] claim1 [to 15].

28. (Amended) A transgenic plant containing a DNA [sequence] molecule according to claim 16 [or 17].

29. (Amended) A method of producing a PUFA comprising carrying out an elongase reaction catalysed by a polypeptide according to [any one of claims] claim 1 [to 15].

32. (Amended) A PUFA produced by a method according to [any one of claims] claim 29 [to 31].

35. (Amended) A pharmaceutical composition comprising a polypeptide according to [any one of claims] claim 1 [to 15].

37. (Amended) A pharmaceutical composition according to claim 35 [or claim 36] wherein the composition comprises a pharmaceutically-acceptable diluent, carrier, excipient or extender.

38. (Amended) A method of elevating the PUFA levels of an animal or a plant comprising the step of [by] supplying to the animal or plant a polypeptide according to [any of claims] claim 1 [to 15, a DNA sequence according to claim 16 or 17, a foodstuff according to claim 33, a dietary supplement according to claim 34, a pharmaceutical composition according to any of

claims 35 to 37 or a PUFA according to claim 32].

39. (Amended) A method [of treatment] according to claim 38 wherein the animal is a mammal.

40. (Amended) A method [of treatment] according to claim 39 wherein the mammal is a human.

FOR EXAMINATION PURPOSES

POLYSATURATED FATTY ACID (PUFA) ELONGASE FROM CAENORHABDITIS ELEGANS

The present invention relates to polyunsaturated fatty acid (PUFA) elongases. More specifically, the invention relates to a DNA sequence from *C. elegans* encoding a PUFA elongase.

Unsaturated fatty acids are essential components required for normal cellular function, being involved in a diverse number of roles ranging from membrane fluidity to acting as signal molecules (Gill, I., Valivety, R. (1997). *Trends Biotechnol.* **15**, 401-409; Broun, P., *et al* (1999) *Ann. Rev. Nutr.* **19**, 197-216). In particular, the class of fatty acids known as the polyunsaturated fatty acids (PUFAs) has attracted considerable interest as pharmaceutical and nutraceutical compounds (Broun *supra*; Horrobin, D. F. (1990) *Reviews in Contemp Pharmacotherpy* **1**, 1-45).

The synthesis of PUFAs i.e. fatty acids of 18 carbons or more in length and containing two or more double bonds, is thought to be catalyzed in a variety of organisms by a specific fatty acid elongase enzyme. This elongase is responsible for the addition of 2 carbon units to an 18 carbon PUFA, resulting in a 20 carbon fatty acid. An example of this reaction is the elongation of γ-linolenic acid (GLA; $18:3\Delta^{6,9,12}$) to di-homo-γ-linolenic acid (DHGLA; $20:3\Delta^{8,11,14}$) in which the tri-unsaturated 18 carbon fatty acid is elongated by the addition of a two carbon unit to yield the tri-unsaturated 20 carbon fatty acid. Since there is considerable interest in the production of long chain PUFAs of more than 18 carbons in chain length, for example arachidonic acid and eicosapentanoic acid, the identification of this enzyme is of both academic and commercial interest.

At present, there are no examples of identified cloned genes encoding PUFA elongases, though a number of genes encoding enzymes likely to be involved in other aspects of lipid synthesis have been identified. For example, an *Arabidopsis* gene (FAE1) has been shown to be required for the synthesis of very long chain monounsaturated fatty acids (such as erucic acid; $20:1\Delta^{11}$) (James, D. W. *et al,* (1995) *Plant Cell* 7, 309-319). However, it is clear that this enzyme does not recognize di- and tri-unsaturated 18 carbon fatty acids, for example, linoleic acid, $18:2\Delta^{9,12}$ or α-linolenic acid, $18:3\Delta^{9,12,15}$ respectively, as substrates,

and is therefore not involved in the synthesis of long chain PUFAs (Millar & Kunst (1997), *Plant Journal* **12**, 121-131). This in itself is not surprising, since, of the plant kingdom, only a very few lower plant species, such as the moss *Physcomicotrella patens* (Girke *et al.*, (1998), *Plant J,* **15**: 39-48); are capable of synthesising long chain PUFAs, and therefore *Arabidopsis* would not be expected to contain any such enzymes (Napier *et al.* (1997), Biochem J, **328**: 717-720; Napier *et al.*, (1999) *Trends in Plant Sci* **4**, 2-5).

A schematic diagram representing a generalized pathway for the product of PUFAs is shown in Figure 1. Biochemical characterisation of mammalian elongation systems (most notably from liver microsomes) has indicated that a mammalian elongase consists of four subunits, made up of a condensing enzyme, a β-ketoreductase, a dehydrase and an enoyl reductase (reviewed in Cinti, D. L., *et al* (1992) *Prog. Lipid Res.* **31**, 1-51). The *Arabidopsis FAE1* gene product encodes a polypeptide of 56kDa, which shows very limited homology to condensing enzymes such as chalcone synthase and stillbene synthase (James, D. W. *supra*). Although *FAE1* is normally only expressed in seed tissues, ectopic expression in non-seed tissue (or heterologously in yeast) revealed that *FAE1* could direct the synthesis of erucic acid (Millar, A. A., Kunst, L. (1997) *Plant J.* **12,** 121-131).

Three fatty acid elongase activities have been characterised from the yeast *S. cerevisiae.* Again, this organism does not synthesis PUFAs, and therefore does not contain genes encoding a PUFA elongase. One gene ELO1, was identified on the basis of a screen to isolate mutants defective in elongation of 14 carbon (i.e. medium) chain saturated fatty acids (Toke & Martin (1996) *J Biol Chem* **271**, 18413-18422). Complementation of *elo1* mutants restored viability, and the ELO1 gene product was shown to encode a polypeptide which was responsible for the specific elongation of 14:0 fatty acids to 16:0 fatty acids.

Two related genes were also detected in the genome of *S. cerevisiae*, and their function determined by disruption. These two genes, subsequently named ELO2 and ELO3, were shown to be involved in the elongation of the very long chain saturated fatty acids found in sphingolipid molecules (Oh *et al* (1997), *J. Biol Chem* **272**, 17376-17384). In particular, ELO2 was required for elongation of fatty acids up to 24 carbons, and ELO3 was required for elongation of the 24 carbon fatty acid to 26 carbons. However, neither gene was

essential for viability. Examination of the these three fatty acid elongases revealed the presence of a conserved "histidine box" motif (Shanklin *et al.*, (1994), *Biochemistry*, **33**, 12787-12794) (His-X-X-His-His, where X is any amino acid) towards the centre of the polypeptide sequences. Importantly, there was no detectable homology between the yeast elongases (ELO1,2,3) and the plant very long chain mono-unsaturated fatty acid elongase (FAE1) (Oh *et al, supra*).

In order to identify genes encoding PUFA elongases, it is necessary to study systems in which the synthesis of PUFAs is well documented; a good example of this is the model animal system *C. elegans*, a small free-living worm (Tanaka *et al.*, (1996), *Lipids* **31**, 1173-1178). *C. elegans*, like most other animals, and in contrast to higher plants, synthesises PUFAs such as arachidonic acid (AA; 20:4 $\Delta^{5,8,11,14}$) as precursors to a class of molecules known as the eicosanoids, which in turn serve as precursors for compounds such as prostaglandins and leucotrienes (Horrobin, (1990), *Reviews in Contemp Pharmacotherpy*, **1**:1-45). The presence of AA and other long chain polyunsaturated fatty acids in *C. elegans* is well documented (Tanaka *et al.*, (1996), *Lipids* **31**, 1173-1178). The complete sequence of the nematode's genome is now publicly available *(The C. elegans consortium, 1998, Science* ***282****, 2012-2018: Database at http://www.sanger.ac.uk/Projects/C_elgans/blast_server.shtml).*

An object of the invention is to provide an isolated PUFA elongase.

Using the above-mentioned *C. elegans* genomic sequence, together with suitable search strings, the inventors identified eight related putative open reading frames (ORFs) encoding for PUFA elongases. A number of different search criteria were applied to identify a number of (ORFs) which were likely to encode polypeptides with fatty acid elongase activities. These ORFs were then subject to functional characterisation by heterologous expression in yeast, allowing the identification of a PUFA elongase.

Accordingly, a first aspect of the invention provides an isolated polypeptide comprising a functional long chain polyunsaturated fatty acid (PUFA) elongase i.e. the polypeptide has the function of extending the chain length of an 18 carbon PUFA to 20 carbons in length.

This polypeptide can be used to elevate PUFA levels in animals, thereby providing a ready source of PUFAs.

The polypeptide may be from a eukaryote.

The polypeptide may comprise at least a portion of the amino acid shown in SEQ ID. 15, or variants thereof.

For the purposes of the present application, the term "variant" in relation to a certain sequence means a protein or polypeptide which is derived from the sequence through the insertion or deletion of one or more amino acid residues or the substitution of one or more animo acid residues with amino acid residues having similar properties, e.g. the replacement of a polar amino acid residue with another polar amino acid residue, or the replacement of a non-polar amino acid residue with another non-polar amino acid residue. In all cases, variants must have an elongase function as defined herein.

A second aspect of the invention provides a polypeptide having at least 60 % homology to a polypeptide according to a first aspect of the invention. The polypeptide may have at least 80%, or as much as 90% or more homology to a polypeptide according to a first aspect of the invention.

The polypeptide according to either aspect of the invention may include a sequence motif responsible for Endoplasmic Reticulum (ER) - retention. This allows the polypeptide to be specifically located or targeted to the ER of a cell.

The polypeptide may also be able to elongate palmitoleic acid (PA; 16:1Δ^9) to vacceric acid (VA; 18:1Δ^{11}). Thus, the polypeptide is also capable of elongation of a Δ^9- monounsaturated 16C fatty acid.

Preferably, the polypeptide is from an animal, more preferably, the animal is an invertebrate such as a worm. Where the animal is a worm, it is preferably *C. elegans*. Alternatively, the animal is a vertebrate, preferably a mammal such as a human, rat or mouse.

A third aspect of the invention provides an isolated DNA sequence, preferably a cDNA sequence, encoding a polypeptide according to a first or second aspect of the invention. This DNA sequence may be used to engineer transgenic organisms.

Preferably, the DNA sequence comprises the sequence shown in SEQ ID NO: 7 or variants of that sequence due, for example, to base substitutions, deletions, and/or additions.

A fourth aspect of the invention provides an engineered organism, such as a transgenic animal, engineered to express a polypeptide according to a first or second aspect of the invention. The engineered organism may be engineered to express elevated levels of the polypeptide, thereby providing a supply of polypeptide at a reduced cost as a reduced number of organisms need be used.

Preferably, the engineered organism is a mammal such as a rat, mouse or monkey.

A fifth aspect of the invention provides an engineered organism containing a synthetic pathway for the production of a polypeptide according to a first or second aspect of the invention. This has the advantage of allowing greater control over the production of PUFAs by the pathway by an organism.

The pathway may include Δ^5-fatty acid desaturase, and/or Δ^6-fatty acid desaturase.

The engineered organism according to a fourth or fifth aspect of the invention may be a lower eukaryote, such as yeast. Alternatively, the transgenic organism may be a fish.

A sixth aspect of the invention provides a transgenic plant engineered to express a polypeptide according to a first aspect of the invention.

A seventh aspect of the invention provides a transgenic plant containing a DNA sequence according to a third aspect of the invention.

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An eighth aspect of the invention provides a method of producing a PUFA comprising carrying out an elongase reaction catalysed by a polypeptide according to a first or second aspect of the invention.

The PUFA may be di-homo-gamma-linoleic acid ($20:3\Delta^{8,11,14}$), arachidonic acid ($20:4\Delta^{5,8,11,14}$), eicosapentanoic acid ($20:5\Delta^{5,8,11,14,17}$), docosatrienoic acid ($22:3\Delta^{3,16,19}$), docosatetraenoic acid ($22:4\Delta^{7,10,13,16}$), docosapentaenoic acid ($22:5\Delta^{7,10,13,16,19}$) or docosahexaenoic acid ($22:6\Delta^{4,7,10,13,16,19}$).

The PUFA may be a 24 carbon fatty acid with at least 4 double bonds.

A ninth aspect of the invention provides a PUFA produced by a method according to an eighth aspect of the invention.

The PUFA may be used in foodstuffs, dietary supplements or pharmaceutical compositions.

A tenth aspect of the invention provides a foodstuff comprising a PUFA according to a fifth aspect of the invention. The foodstuff can be fed to an animal.

An eleventh aspect of the invention provides a dietary supplement comprising a PUFA according to a fifth aspect of the invention. The dietary supplement can be supplied to an animal to augment its PUFA levels.

An twelfth aspect of the invention provides a pharmaceutical composition comprising a polypeptide according to a first or second aspect of the invention or a PUFA according to a ninth aspect of the invention.

Preferably, the pharmaceutical composition comprises a pharmaceutically-acceptable diluent, carrier, excipient or extender. This allows the composition to be supplied in a form which best suits the pharmaceutical application in question. For example, a topical application would preferably be a cream or lotion, whereas if the composition was to be ingested a different form would be more suitable.

A thirteenth aspect of the invention provides a method of treatment of an animal, such as a mammal, or a plant, comprising supplying to the animal or plant a DNA sequence according to a third aspect of the invention, a foodstuff according to a tenth aspect of the invention, a dietary supplement according to an eleventh aspect of the invention, a pharmaceutical composition according to a twelfth aspect of the invention or a PUFA according to a ninth aspect of the invention.

Preferably, the mammal is a human.

The invention will now be further described, by way of example only, with reference to SEQ ID1 to 16, and Figures 2 to 11, in which;

SEQ ID1 to 8 show the putative ORFs encoding PUFA elongases A to H respectively; and

SEQ ID9 to 16 show the deduced amino acid sequences of the putative ORFs of SEQ ID NO: 1 to 8 respectively; and

Figures 2 to 9 show hydrophobicity plots for each of PUFA elongases A to H respectively.

Figure 10 shows an amino acid sequence line-up comparing the *C. elegans* ORF F56H11.4 (Z68749) with related sequences.

Figure 11 shows chromatograms of fatty acid methyl esters from transformed yeast.

Introduction to general strategy

Initially the *C. elegans* databases were searched for any sequences which showed low levels of homology to yeast ELO genes (*ELO2* and *ELO3*) using the TBLASTN programme. A similar search was carried out using short (20 to 50 amino acid) stretches of ELO genes which were conserved amongst the three ELO polypeptide sequences. *C. elegans* sequences which were identified by this method were then used themselves as search probes, to identify any related *C. elegans* genes which the initial search with the yeast sequences failed to identify. This was necessary because the level of homology between the yeast ELO genes

and any worm genes is always low (see BLAST scores later). To allow for a more sensitive search of worm sequences, a novel approach was adopted to circumvent the major drawback with searches using the BLAST programmes, namely that the search string (i.e. the input search motif) must be longer than 15 characters for the algorithm to work. Thus, if it was desired to search for a short motif (like a histidine box), then the BLAST programme would not be capable of doing this. A complete list of all the predicted ORFs present in the *C. elegans* genome exists as a database called Wormpep, which is freely available from the Sanger WWW site (http://www.sanger.ac.uk/Projects/C_elegans/webace_front_end.shtml). The latest version of Wormpep was down loaded to the hard disc of a Pentium PC, and re-formatted as a Microsoft Word6 document, resulting in a document of about 3,500 pages. This was then searched using the "Search & Replace" function of Word6, which also allows for the introduction of "wildcard" characters into the search motif. So, for example, it is possible to search both for the short text string HPGG, which would identify any predicted worm ORF present in the Wormpep 3,500 page document containing this motif, or alternatively search with HPGX (where X is a wild card character). Clearly, such (manual) searches of a 3,500 page document are extremely time-consuming and demanding, also requiring visual inspection of each and every identified ORF. For example, searching with a motif such as HXXHH identifies in excess of 300 different ORFs. However, by using a number of different short search strings (as outlined below), and combining these with other methods for identifying putative elongase enzymes, a number of candidate ORFs have been identified.

Database search using the FAE1 polypeptide sequence

As a negative control, to demonstrate that the FAE1 gene sequence was unlikely to provide a useful search sequence in the identification of *C.elegans* sequences encoding for PUFA elongases, the GenBank databases (http://www.ncbi.nlm.nih.gov/Web/Search/index.html) were searched using the *Arabidopis* FAE1 polypeptide sequence to identify related genes or expressed sequence transcripts (ESTs). GenBank is the NIH genetic sequence database, an annotated collection of all publicly available DNA sequences (*Nucleic Acid Research* (1998) **26**, 1-7). There are approximately 2,162,000,000 bases in 3,044,000 sequence records as of December 1998. The search was carried out using the BLAST2 (Basic Local Alignment Search Tool) algorithm (Altschul *et al.*, (1990) *J Mol Biol* **215**,403,410) Although a number

of plant ORFs and ESTs were reported as being related, no animal sequences were identified by this search, confirming the observation that FAE1 was unlikely to be a suitable candidate as a search template for PUFA elongases.

Database search using yeast ELO sequences

Using the three yeast fatty acid elongase sequences (ELO 1, 2, 3) as probes, a number of putative ORFs in the DNA of *C. elegans*-derived cosmid sequences which form the *C. elegans* genomic sequence database were identified. Moreover, an extensive and time-consuming search of a downloaded copy of the WormPep database (ftp://ftp.sanger.ac.uk./pub/databases/wormpep) using manual search strings in MSWord 6, identified a number of *C. elegans* ORFs which contained presumptive histidine boxes. Wormpep contains predicted proteins from the *Caenorhabditis elegans* genome sequence project, which is carried out jointly by the Sanger Centre in Cambridge, UK and Genome Sequencing Center in St. Louis, USA. The current Wormpep database, Wormpep 16, contains 16,332 protein sequences (7,120,115 residues). Search strings used included [HXXHH], [HXXXHH], [QXXHH] and [YHH]. Comparison of the data from the two different searches indicated a small (<10) number of putative ORFs as candidate elongases. The histidine box motifs are shown in bold in SEQ ID 9 to 16.

Hydrophobicity plot analysis

Since the fatty acid elongase reaction is predicted to be carried out on the cytosolic face of the endomembrane system (Toke & Martin (1996), *supra*; Oh *et al* (1997), *supra)*, the putative *C. elegans* ORFs were examined for potential membrane spanning domains, via Kyte & Doolittle hydrophobicity plots *(J. Mol Biol,* (1982), **157**, 105-132). This revealed a number of ORFs with possible membrane-spanning domains, and also indicated a degree of similarity in the secondary-structure of a number of identified ORFs.

Screening for ER-retention signal sequences

The inventors postulated that since fatty acid elongases are expected to be endoplasmic reticulum (ER) membrane proteins, they might be expected to have peptide signals which are responsible for "ER-retention". In the case of ER membrane proteins, this signal often takes the form of a C-terminal motif [K-K-X_{2-3}-Stop], or similar variants thereof (Jackson *et*

al., (1990), *EMBO J.*, **9**, 3153-3162). Further sequence analysis of the *C. elegans* putative elongases revealed that 4 ORFs (F41H10.7, F41H10.8, F56H11.4, Y53F4B.c) had *C*-terminal motifs that exactly matched this search pattern, and that a further 2 ORFs (F11E6.5, C40H1.4) had related sequences. These sequence motifs are underlined in SEQ ID 9 to 13, 15 and 16.

Chromosome mapping

Since the inventors had previously observed that *C.elegans* genes involved in the synthesis of PUFA may exist in tandem (for example the Δ5 and Δ6 desaturases required for AA and GLA synthesis, respectively, are < 1 kB apart on chromosome IV (Michaelson *et al.*, (1998), *FEBS Letts* **439**, 215-218), the positions of the putative *C. elegans* elongase ORFs were determined using the Sanger Centre's WebAce *C. elegans* server (http://www.sanger.ac.uk/Projects/C_elegans/webace_front_end.shtml).. This indicated that two pairs of putative elongases were in close proximity to each other on the *C. elegans* chromosome IV.

F41H10.7 and F41H10.8 were identified as being approximately 10 Kb apart on chromosome IV, and F56H11.3 and F56H11.4 were identified as being approximately 2 Kb apart on chromosome IV.

Putative *C. elegans* fatty acid elongases

The positions of the putative ORFs in the *C. elegans* genome are shown below i.e. chromosome number, and map position in centiMorgans, together with the GenBank database accession numbers.

The designations used employ the same method as used on the Sanger Centre's *C. elegans* database, i.e. ORF C40H1.4 is predicted coding sequence 4 on cosmid C40H1.

Elongase	Cosmid Sanger ID Code	GenBank Acc	Chromosome
A	C40H1.4	Z19154	III

B	D2024.3	U41011	IV, 7.68
C	F11E6.5	Z81058	IV, 18.8
D	F41H10.7*	U61954	IV, 29.8
E	F41H10.8*	U61954	IV, 29.8
F	F56H11.3#	Z68749	IV, 2.5
G	F56H11.4#	Z68749	IV, 2.5
H	Y53F4B.c	Z92860	II

* or# indicates genes in tandem

Comparison of *C. elegans* putative elongase ORFs with yeast genes:

Each of the three yeast ELO polypeptides were compared against all of the worm putative elongase translated ORF sequences, and then ranked in order of similarity (as measured by the BLAST score) (Altschul *et al* (1990), *supra*)

The results are shown below, with the ORF sequences ranked from most similar to least similar, and the BLAST scores are shown in brackets:

Yeast ELO1 (14 to 16 carbon fatty acid elongase)

G (262) > E (241) > D (225) > C (219) > A (216) > F (215) > H (197) > B (172)

Yeast ELO2 (24 carbon sphingolipid elongase)

E (231) > C (226) > G (189) > A (181) > F (166) > D (150) > H (141) > B (140)

Yeast ELO3 (24 to 26 sphingolipid elongase)

D (171) > G (163) > F (154) > A (152) > E (150) > C (131) > B (132) > H (128)

It is clear from the numeric values of the BLAST scores that the sequences are related, but the levels of homology are low. For comparison, the BLAST score for homology between two related worm proteins, the Δ5 and the Δ6 desaturase is in excess of 500.

Analysis of potential sphingolipid ancestry

Previously, the inventors had noted the similarities between the fatty acid Δ6 desaturase and sphingolipid desaturases in plants, and that the two distinct enzymes could have arisen from one ancestral gene. Moreover, it was considered likely that the sphingolipid desaturase predated the fatty acid desaturase, and may in fact have been the ancesteral progenitor. Therefore it is plausible that the next step in the arachidonic acid biosynthetic pathway has also evolved from the sphingolipid metabolic pathway. It is therefore considered highly significant that some of the *C. elegans* ORF putative elongases have similarity to sphingolipid enzymes. For this reason, these ORFs are considered to be very clear candidates for PUFA elongases. It has previously been considered that the *C. elegans* Δ5 and Δ6 fatty acid desaturases have evolved from 1 ancestral gene (Michaelson *et al.*, (1998), *FEBS Letts* **439**, 215-218). It is also significant that one pair of *C. elegans* putative elongase ORFs (F & G) genetically maps close to the Δ5/Δ6 fatty acid desaturase genes, with both gene pairs being located at the top end of chromosome IV.

Cosmid Sanger ID Code	GenBank Acc	Chromosome	Encoded Peptide
W08D2.4	Z70271	IV, 3.06	Δ6 fatty acid desaturase
T13F2.1	Z81122	IV, 3.06	Δ5 fatty acid desaturase

Cloning of Desaturase and Elongase Genes in Yeast Expression Vectors

Putative elongases sequences F56H11.4 and F41H10.8 were cloned by PCR into the pYES2 vector (Invitrogen). A *C. elegans* mixed stage cDNA library was used as a PCR template. F56H11.4 was amplified using primers:

56h114.for 5'-GCGGGTACCATGGCTCAGCATCCGCTC-3' and;

56h114.rev 5'-GCGGGATCCTTAGTTGTTCTTCTTCTT-3'.

F41H10.8 was amplified using primers:

41h108.for 5'-GCGGGTACCATGCCACAGGGAGAAGTC-3' and;

41h108.rev 5'-GCGGGATCCTTATTCAATTTTTCTTTT-3'.

Amplified sequences were then restricted using *KpnI* and BamHI (underlined in the forward and reverse primers, respectively), purified using the Qiagen PCR purification kit, and ligated into a KpnI/BamHI cut pYes2 vector.

An ORF encoding the *Mortierella alpina* Δ^5-fatty acid desaturase (Michaelson, L. V., *et al* (1998) *J. Biol. Chem.* **273**, 19055-19059) was amplified using primers:

Mad5.for 5'-GCGAATTCACCATGGGTACGGACCAAGGA-3' and;

Mad5.rev 5'-GCGGAGCTCCTACTCTTCCTTGGGACG-3',

and restricted using *Eco*RI and *Sac*I, gel purified as described and ligated into a *Eco*RI/*Sac*I cut pESC-TRP vector (Stratagene) to generate pESC/Δ^5.

An ORF encoding the borage Δ^6-fatty acid desaturase (Sayanova, O., *et al* (1997) *Proc. Natl. Acad. Sci USA* **94**, 4211-4216) was restricted from pGEM3 using *Bam*HI and *Xho*I and ligated into a *Bam*HI/*Xho*I cut pESC-TRP vector to generate pESC/Δ^6.

A double construct was also generated by ligating the *Bam*HI/*Xho*I borage Δ^6 insert into the pESC/Δ^5 construct described previously, generating pESC/(Δ^5,Δ^6).

Functional Characterisation in Yeast

Elongases and desaturase constructs were introduced in *Saccharomyces cerevisiae* W303-1A using a lithium acetate based method (Elble, R. (1992) *Biotechniques* **13**, 18-20) and expression of the transgenes was induced by addition of galactose to 2% (w/v) as described in Napier *et al* (Napier, J. A., *et al* (1998) *Biochem J* **330**, 611-614; Michaelson L. V., *supra*; Michaelson, L. V., (1998) *FEBS Letts* **439**, 215-218). Yeast transformants containing pYES2-derived constructs were grown on synthetic minimal media (SD, the composition of which is defined in Sherman, F (1991) *Methods in Enzymology* **194**, 3-21); synthetic minimal medium minus uracil; pESC-derived constructs were grown on SD minimal medium minus tryptophan. Co-transformed yeast (containing both pYES2 and pESC derivatives) were grown on SD minimal medium minus uracil and tryptophan. Prior to induction, cultures were grown in the presence of 2% raffinose and supplemented with 0.5 mM of the appropriate fatty acid substrate in the presence of 1% tergitol-(NP40) (Sigma). All cultures were then grown for a further 48-h unless indicated.

Fatty Acid Analysis

To identify the elongation reaction responsible for the synthesis of di-homo-γ-linolenic acid (DHGLA; 20:3$\Delta^{8,11,14}$) from GLA, this latter fatty acid was supplied as the (exogenous) substrate.

Lipids were extracted from transformed and control yeast by homogenisation in MeOH-$CHCl_3$ using a modification of the method of Bligh and Dyer (Dickenson & Lester (1999) *Biochim Biophys Acta* **1426**, 347-357). The resulting $CHCl_3$ phase was evaporated to dryness under nitrogen gas and the samples were transmethylated with 1M HCl in methanol at 80 °C for 1 hour. Fatty acid methyl esters (FAMES) were extracted in hexane and purified using a small column packed with Florisil. Analysis of FAMES was conducted using a Hewlett Packard 5880A Series Gas Chromatograph equipped with a 25M x 0.32mm RSL-500BP bonded capilliary column and a flame ionisation detector. Fatty acids were identified by comparison of retention times with FAME standards (Sigma)

separated on the same GC. Quantitation was carried out using peak height area integrals expressed as a total of all integrals (Bligh, E.G. & Dyer, W.J. (1959) *Can. J. Biochem. Physiol.* **37**, 911-917).

Total fatty acids extracted from yeast cultures were analysed by gas chromatography (GC) of methyl ester derivatives. Lipids were extracted, transmethylated and the fatty acid methyl esters (FAMEs) analysed as described by Sayanova *et al.*

Figure 11 shows chromatograms of fatty acid methyl esters from yeast transformed with the control (empty) plasmid pYES2 (Fig. 11A) or with ORF F56H11.4 in pYES2 (Fig. 11B). Exogenous substrate in the form of GLA was supplied to the cultures. Two novel peaks are observed in (B); these peaks (annotated as 20:3 and 18:1*) were identified (against known standards) as DHGLA and vaccenic acid, respectively. Detection was by flame ionisation.

One cDNA ORF tested in this manner displayed a high level of elongase activity on the GLA substrate, converting 44% to DHGLA. The identity of this elongation product was confirmed as DHGLA by comparison with a known standard (the standards used were known standards for either DHGLA, AA, EPA or VA from Sigma Chemicals, Ltd.), using GCMS analysis using a Kratos MS80RFA (Napier, J. A., *supra*; Michaelson, L. V., *supra*; Michaelson, L. V., *supra*). The deduced amino acid sequence of the functional elongase clone identified it as being encoded by the *C. elegans* gene F56H11.4, and comparison with the yeast *ELO* genes showed low homology confined to a few short amino acid motifs (see Fig. 10). Some similarity with a mouse gene Cig30 (Tvrdik, P., (1997) *J. Biol. Chem.* **272**, 31738-31746), which has been implicated in the recruitment of brown adipose tissue in liver tissue, was also observed, as well as a potential human homologue encoded by a gene located on chromosome 4q25, BAC 207d4. The most closely related *C. elegans* ORFs, F41H10.8 (U61954) and F56H11.3 (Z68749) are also shown, as is part of a related human gene present on chromosome IV (present on BAC clone B207d4; AC004050). The GenBank accession numbers are given for all sequences.

The range of fatty acids synthesised by *C. elegans* can potentially require a number of different elongation reactions (Tanaka, T., (1996) *Lipids* **31**, 1173-1178). The substrate-specificity of the F56H11.4 PUFA elongase was therefore determined using a

range of exogenously supplied fatty acids. This revealed that GLA is the major substrate, with a number of other fatty acids being elongated at a lower efficiency (see Table 1). Although most of these substrates are polyunsaturated fatty acids, it was unexpectedly observed that palmitoleoic acid (PA; 16:1 Δ^9) was also elongated by F56H11.4 to yield vaccenic acid (VA; 18:1 Δ^{11}). The biosynthetic pathway for VA is unclear, but the data indicate that it may be synthesised by elongation of Δ^9-monounsaturated 16C fatty acid.

The *C. elegans* PUFA elongase ORF F56H11.4 maps to the top of chromosome IV (at 4.32 cM) with a related sequence (F56H11.3; 51 % similarity) located 1,824bp downstream. Another *C. elegans* gene (F41H10.8) was also observed, which is present on chromosome IV, and which shows a slightly higher level (53%) of similarity to the PUFA elongase than F56H11.3 (see Fig. 10). However, when a PCR product encoding ORF F41H10.8 was expressed in yeast in a manner identical to that used for F56H11.4, the former failed to direct the elongation of any fatty acids, despite the provision of a range of substrates (see Table II).

In order to reconstitute the PUFA biosynthetic pathway in a heterologous system, the PUFA elongase F56H11.4 was expressed in yeast in conjunction with either the Δ^6- or Δ^5-fatty acid desaturases previously isolated and characterised by the inventor (Napier, J. A., *supra*; Michaelson, L. V., *supra*). Expression of the Δ^6-fatty acid desaturase and F56H11.4 was carried out in the presence of two different substrates (LA or ALA) while the Δ^5-fatty acid desaturase and the elongase were expressed in the presence of GLA only. This demonstrated that was possible to combine a desaturase and an elongase in yeast to generate significant amounts of a final "product" (see Table III). In the case of the elongase and the Δ^6-fatty acid desaturase, the reactions proved highly efficient with the production of 4.5% of DHGLA from the LA substrate. This resulted from 25% desaturation of the LA substrate to GLA, which was then elongated to DHGLA at a similar level of efficiency (18%). This is lower than the % conversion observed for GLA when supplied exogenously (see Table I), indicating that the *in vivo* production of substrates for elongation may be rate-limiting.

If ALA was used as a substrate, 27% of this was initially Δ^6-desaturated to yield octadecatetraenoic acid (OTA; 18:4 $\Delta^{6,9,12,15}$) but only 8% of was subsequently elongated to yield eicosatetraenoic acid (20:4 $\Delta^{8,11,14,17}$). Thus, the conversion efficiency of ALA to the final 20-carbon tetraenoic PUFA was only about 2.2%.

Since DHGLA is an *n-6* fatty acid, whilst the OTA-derived eicostetraenoic acid is an *n-3* type, this demonstrates that the elongase is capable of accepting both forms of essential fatty acid, albeit with different efficiencies. Verification was also provided that the 20C PUFAs synthesised in the yeast expression system were generated by the Δ^6-desaturation of 18C substrates which were subsequently elongated, as the Δ^6-desaturase showed no activity on 20:2 or 20:3 substrates (see Table III).

The combination of the Δ^5-desaturase and the elongase also demonstrated that these two enzymes could work in tandem, although the efficiency of this overall conversion was lower (3.3% AA from GLA) which was due to the previously observed low activity of the Δ^5-desaturase enzyme itself (Michaelson, L. V., *supra*; Michaelson, L. V., *supra*). Thus, although nearly 45% of the GLA substrate was elongated to DHGLA, only 7.5% of this was then desaturated to AA (see Table III).

Finally, the production of either AA or eicosapentanoic acid (EPA; 20:5$\Delta^{5,8,11,14,17}$) in yeast from dienoic or trienoic 18 carbon substrates was achieved via expression of all three enzymes (the two desaturases and the F56H11.4 PUFA elongase) simultaneously. As shown in Table IV, small but significant amounts of AA were produced when the yeast was supplied with the 18C dienoic fatty acid LA.

GC-Mass Spectroscopy (MS) Analysis

Peak identification and confirmation were carried out by GC-MS using a Kratos MS80RFA using known standards (Sigma). The identity of this 20C PUFA was verified by GCMS, indicating that the conversion efficiency from LA was 0.65%. When ALA was used as a substrate, 12.5% of the (Δ^6-desaturated and elongated) eicosatetraenoic *n-3* fatty acid was Δ^5-desaturated, resulting in a total conversion of 0.3% of the ALA substrate to EPA (the identity of EPA was confirmed by GCMS).

Expression of *C. elegans* elongase in plants

In order to express *C. elegans* elongase in plants, the following protocol is an example of a process which can be used to create the transgenic plants. *C. elegans* ORF sequence can be subcloned into a plant expression vector pJD330, which comprises a viral 35S promoter, and a Nos terminator. The resulting cassette or promoter/coding sequence/terminator can then be subcloned into the plant binary transformation vector pBin 19, and the resulting plasmid introduced into *Agrobacterium tumefaciens*. This *Agrobacterium* strain can then be used to transform Arabidopsis by the vacuum-infiltration of inflorescences, and the seeds harvested and plated onto selective media containing kanamycin. Since pBin 19 confers resistance to this antibotic, only transformed plant material will grow. Resistant lines can therefore be identified and self-fertilized to produce homozygous material. Leaf material can then be analyzed for expression of *C. elegans* elongase.

Fatty acid methyl ester analysis can be carried out as previously described.

Table I

ORF	-	*mole% Fatty Acids* F56H11.4									
	(Control)										
Substrate	-	-		GLA		LA		ALA		EPA	
Induction	-	+	-	+	-	+	-	+	-	+	-
16:0	17.5 ± 3.3	19.9 ± 3.5	20.5 ± 4.1	27.7 ± 1.4	29.8 ± 0.2	22.9 ± 1.5	23.9 ± 1.0	19.1 ± 0.7	20.2 ± 1.1	23.4 ± 0.2	24.2 ± 1.0
16:1	53.2 ± 7.2	40.9 ± 3.1	49.4 ± 3.2	32.5 ± 4.4	34.4 ± 1.8	21.2 ± 2.2	24.8 ± 4.9	18.1 ± 1.5	19.6 ± 2.5	26.9 ± 0.7	34.5 ± 1.5
18:0	4.5 ± 0.7	4.7 ± 0.9	4.9 ± 0.5	5.6 ± 0.5	5.6 ± 0.3	5.1 ± 0.3	4.4 ± 0.1	5.0 ± 0.3	4.5 ± 0.1	5.3 ± 0.2	5.4 ± 0.2
18:1	24.8 ±3.9	24.9 ± 1.4	25.2 ± 2.3	16.9 ± 0.9	16.1 ± 0.3	11.2 ± 2.4	10.7 ± 1.5	10.1 ± 1.1	9.9 ± 1.2	15.4 ± 0.4	17.8 ± 1.1
18:1*	-	9.6 ± 0.6	-	3.9 ± 0.6	-	3.2 ± 0.6	-	3.1 ± 0.4	-	6.2 ± 0.3	-
LA	-	-	-	-	-	34.4 ± 4.2	36.2 ± 5.6	-	-	-	-
ALA	-	-	-	-	-	-	-	43.1 ± 3.9	45.8 ± 4.8	-	-
GLA	-	-	-	7.5 ± 1.2	14.0 ± 0.3	-	-	-	-	-	-
20:2	-	-	-	-	-	2.0 ± 0.9	-	-	-	-	-
DHGLA	-	-	-	5.8 ± 0.9	-	-	-	-	-	-	-
20:3	-	-	-	-	-	-	-	1.5 ± 0.1	-	-	-
EPA	-	-	-	-	-	-	-	-	-	22.8 ± 0.7	20.1 ± 2.4
% Elongated											
GLA	-	-	-	44	-	-	-	-	-	-	-
LA	-	-	-	-	-	5.5	-	-	-	-	-
ALA	-	-	-	-	-	-	-	3.4	-	-	-
EPA	-	-	-	-	-	-	-	-	-	0	-

Table II

mole% Fatty Acids

ORF	F41H10.8									
Substrate	-		GLA		LA		ALA		EPA	
Induction	+	-	+	-	+	-	+	-	+	-
16:0	19.0 ± 0.9	19.3 ± 0.2	28.1 ± 0.6	28.0 ± 0.9	23.9 ± 0.7	24.4 ± 0.2	22.8 ± 0.2	23.4 ± 0.2	23.0 ± 0.6	23.7 ± 0.9
16:1	50.9 ± 0.7	50.8 ± 0.6	33.5 ± 2.2	35.5 ± 1.5	22.4 ± 2.1	23.6 ± 0.3	17.6 ± 0.2	15.8 ± 0.9	34.7 ± 3.6	32.2 ± 3.2
18:0	4.2 ± 0.1	5.1 ± 0.1	5.3 ± 0.1	5.6 ± 0.1	5.1 ± 0.2	5.8 ± 0.1	5.4 ± 0.3	5.9 ± 0.1	4.8 ± 0.7	5.1 ± 0.3
18:1	24.5 ± 1.3	24.9 ± 0.5	16.2 ± 1.4	17.1 ± 1.0	9.1 ± 0.3	10.1 ± 0.2	7.8 ± 0.1	9.5 ± 0.6	15.3 ± 2.5	15.3 ± 1.8
18:1*	ND	-	ND	-	ND	-	ND	-	ND	-
LA	-	-	-	-	39.5 ±0.6	36.1 ± 0.4	-	-	-	-
ALA	-	-	-	-	-	-	46.4 ± 0.5	45.4 ± 1.3	-	-
GLA	-	-	14.3 ± 1.6	14.2 ± 0.6	-	-	-	-	-	-
20:2	-	-	-	-	ND	-	-	-	-	-
DHGLA	-	-	ND	-	-	-	-	-	-	-
20:3	-	-	-	-	-	-	ND	-	-	-
EPA	-	-	-	-	-	-	-	-	22.3 ± 2.8	23.8 ± 2.2
% Elongated										
GLA	-	-	0	-	-	-	-	-	-	-
LA	-	-	-	-	0	-	-	-	-	-
ALA	-	-	-	-	-	-	0	-	-	-
EPA	-	-	-	-	-	-	-	-	0	-

Table III

mole% Fatty Acids

Construct	Δ^6		F56H11.4 + Δ^6				F56H11.4 + Δ^5	
			LA		ALA		GLA	
Substrate	20:2	20:3						
Induction	+	-	+	-	+	-	+	-
16:0	24.7 ± 1.3	25.2 ± 1.5	18.7 ± 0.6	23.7 ± 0.5	17.4 ± 0.7	21.0 ± 1.3	27.9 ± 4.2	29.8 ± 3.8
16:1	46.0 ± 2.8	43.7 ± 3.7	18.9 ± 1.2	24.6 ± 0.7	5.3 ± 0.6	9.1 ± 0.9	24.6 ± 3.4	25.1 ± 3.2
16:2	5.2 ± 1.2	4.1 ± 1.4	0.6 ± 0.1	-	0.4 ± 0.1	-	-	-
18:0	4.8 ± 0.4	5.1 ± 0.4	4.0 ± 0.3	5.1 ± 0.1	6.2 ± 0.7	5.4 ± 0.2	5.6 ± 0.8	5.4 ± 0.7
18:1	15.3 ± 1.1	16.1 ± 1.2	12.2 ± 1.4	11.2 ± 0.4	5.7 ± 0.8	6.0 ± 0.4	12.7 ± 2.9	13.0 ± 2.5
18:1*	-	-	7.7 ± 0.7	-	2.6 ± 0.3	-	2.9 ± 0.9	-
LA	-	-	25.0 ± 3.2	35.4 ± 2.1	-	-	-	-
ALA	-	-	-	-	42.3 ± 3.3	58.5 ± 4.7	-	-
GLA	-	-	7.9 ± 2.2	-	-	-	13.2 ± 3.6	19.2 ± 3.5
OTA	-	-	-	-	15.3 ± 1.8	-	-	-
20:2	4.0 ± 0.3	-	3.3 ± 0.5	-	-	-	-	-
DHGLA	-	-	1.7 ± 0.2	-	-	-	9.8 ± 1.8	-
20:3	-	5.8 ± 0.5	-	-	3.4 ± 0.4	-	-	-
AA	-	-	-	-	-	-	0.8 ± 0.2	-
20:4	-	-	-	-	1.4 ± 0.2	-	-	-
EPA	-	-	-	-	-	-	-	-
% Elongated								
GLA	-	-	17.7	-	-	-	44.5	-
OTA	-	-	-	-	8.4	-	-	-
LA	-	-	8.7	-	-	-	-	-
ALA	-	-	-	-	5.4	-	-	-

SEQ ID1

C40H1.4

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SEQ ID2

D2024.3

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SEQ ID3

F11E6.5

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SEQ ID4

F41H10.7

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caaacaaagaaggctaactaa

SEQ ID5

F41H10.8 (ce477)

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gaagactgaa aagaaaattg aataa

SEQ ID6

F56H11.3

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SEQ ID7

F56H11.4 (Ce 166)

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SEQ ID8

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SEQ ID10

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SEQ ID14

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SEQ ID15

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SEQ ID16

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Replacement Sheet

Claims

1. An isolated polypeptide comprising a functional long chain polyunsaturated fatty acid (PUFA) elongase as herein defined.

2. A polypeptide according to claim 1 wherein the polypeptide is from a eukaryote.

3. A polypeptide according to claim 1 or claim 2 wherein the polypeptide has at least a portion of the amino acid sequence shown in SEQ ID 15, or variants thereof.

4. A polypeptide having at least 60% homology to a polypeptide according to claim 3 and having a PUFA elongase function.

5. A polypeptide according to claim 4 having at least 80% homology.

6. A polypeptide according to claim 5 having at least 90% homology.

7. A polypeptide according to any preceding claim wherein the polypeptide sequence includes a sequence motif responsible for Endoplasmic Reticulum (ER) - retention.

8. A polypeptide according to any preceding claim wherein the polypeptide is capable of elongating palmitoleic acid (PA; $16:1\Delta^{9}$) to vacceric acid (VA; $18:1\Delta^{11}$).

9. A polypeptide according to any preceding claim wherein the polypeptide is from an animal.

10. A polypeptide according to claim 9 wherein the animal is an invertebrate.

11. A polypeptide according to claim 10 wherein the invertebrate is a worm.

12. A polypeptide according to claim 11 wherein the worm is *C. elegans*.

13. A polypeptide according to claim 9 wherein the animal is a vertebrate.

14. A polypeptide according to claim 13 wherein the vertebrate is a mammal.

15. A polypeptide according to claim 14 wherein the mammal is a human, rat or mouse.

16. A DNA sequence encoding a polypeptide according to any preceding claim.

17. A DNA sequence according to claim 16 wherein the DNA comprises the sequence shown in SEQ ID 7 or variants of that sequence due to base substitutions, deletions and/or additions.

18. An engineered organism engineered to express a polypeptide according to any one of claims 1 to 15.

19. An engineered organism according to claim 18 wherein the animal is a mammal.

20. An engineered organism according to claim 19 wherein the mammal is a rat, mouse or monkey.

21. An engineered organism containing a synthetic pathway for the production of a polypeptide according to any one of claims 1 to 15.

22. An engineered organism according to claim 21 wherein the pathway includes Δ^5-fatty acid desaturase.

23. An engineered organism according to claim 21 or 22 wherein the pathway includes Δ^6-fatty acid desaturase.

24. An engineered organism according to any one of claims 21 to 23 wherein the animal is a lower eukaryote.

25. An engineered organism according to claim 24 wherein the lower eukaryote is a yeast.

26. An engineered organism according to claim 18 wherein the animal is a fish.

27. A transgenic plant engineered to express a polypeptide according to any one of claims 1 to 15.

28. A transgenic plant containing a DNA sequence according to claim 16 or 17.

29. A method of producing a PUFA comprising carrying out an elongase reaction catalysed by a polypeptide according to any one of claims 1 to 15.

30. A method according to claim 29 wherein the PUFA is di-homo-gamma-linoleic acid ($20:3\Delta^{8,11,14}$), arachidonic acid ($20:4\Delta^{5,8,11,14}$), eicosapentanoic acid ($20:5\Delta^{5,8,11,14,17}$), docosatrienoic acid ($22:3\Delta^{3,16,19}$), docosatetraenoic acid ($22:4\Delta^{7,10,13,16}$), docosapentaenoic acid ($22:5\Delta^{7,10,13,16,19}$) or docosahexaenoic acid ($22:6\Delta^{4,7,10,13,16,19}$).

31. A method according to claim 29 wherein the PUFA is a 24 carbon fatty acid with at least 4 double bonds.

32. A PUFA produced by a method according to any one of claims 29 to 31.

33. A foodstuff comprising a PUFA according to claim 32.

34. A dietary supplement comprising a PUFA according to claim 32.

35. A pharmaceutical composition comprising a polypeptide according to any one of claims 1 to 15.

36. A pharmaceutical composition comprising a PUFA according to claim 32.

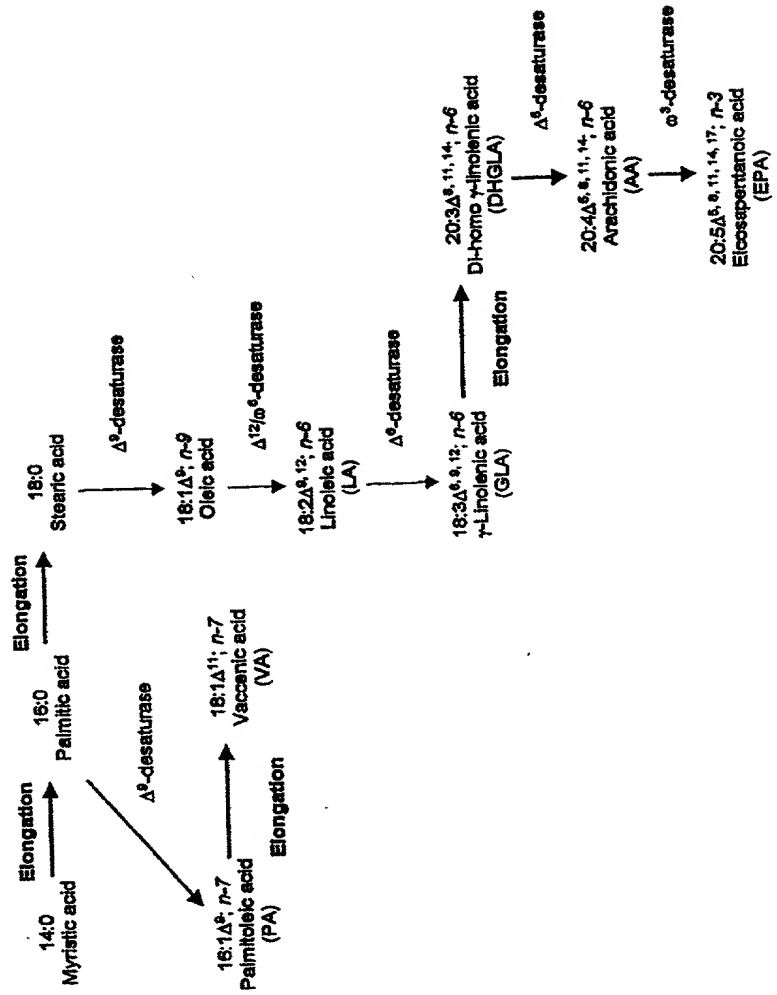
37. A pharmaceutical composition according to claim 35 or claim 36 wherein the composition comprises a pharmaceutically-acceptable diluent, carrier, excipient or extender.

38. A method of elevating the PUFA levels of an animal or a plant by supplying to the animal or plant a polypeptide according to any of claims 1 to 15, a DNA sequence according to claim 16 or claim 17, a foodstuff according to claim 33, a dietary supplement according to claim 34, a pharmaceutical composition according to any of claims 35 to 37 or a PUFA according to claim 32.

39. A method of treatment according to claim 38 wherein the animal is a mammal.

40. A method of treatment according to claim 39 wherein the mammal is a human.

SUBSTITUTE SHEET (RULE 26)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Δ6-desaturase
18:3Δ6,9,12; n-6
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(GLA)
Elongation
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(DHGLA)
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Arachidonic acid
(AA)
ω3-desaturase
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(EPA)
Δ9-desaturase
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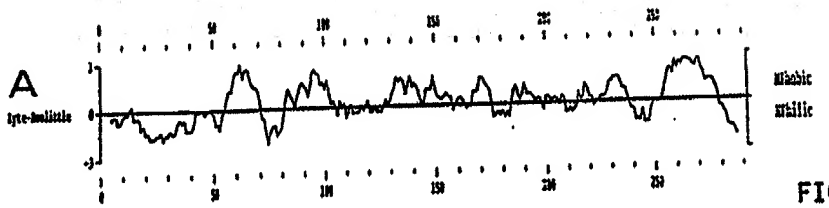
Vaccenic acid
(VA)

FIG. 1

FOBT60 Sh89E660



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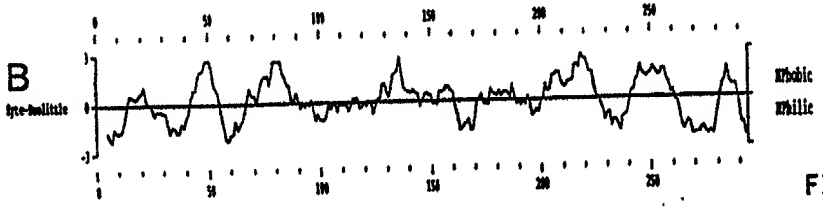

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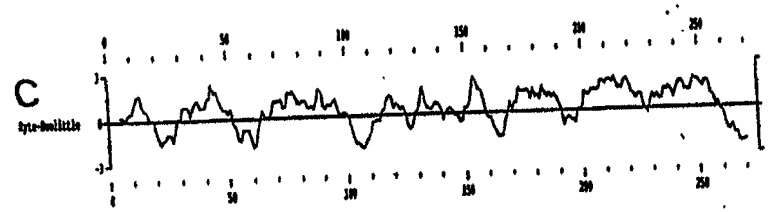

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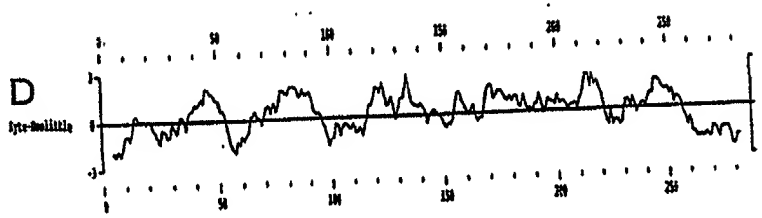


FIG 5

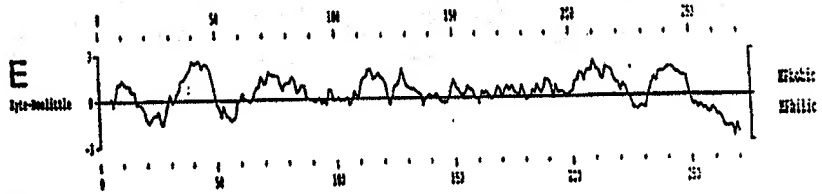
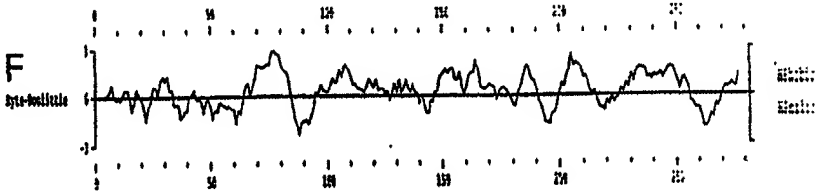


FIG 6



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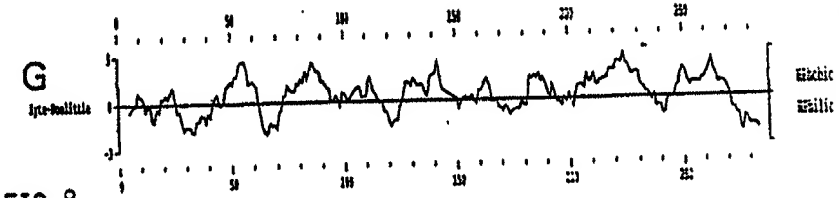

FIG 8

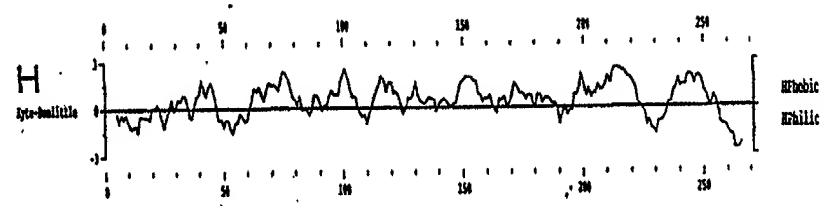

FIG 9

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FIG. 10

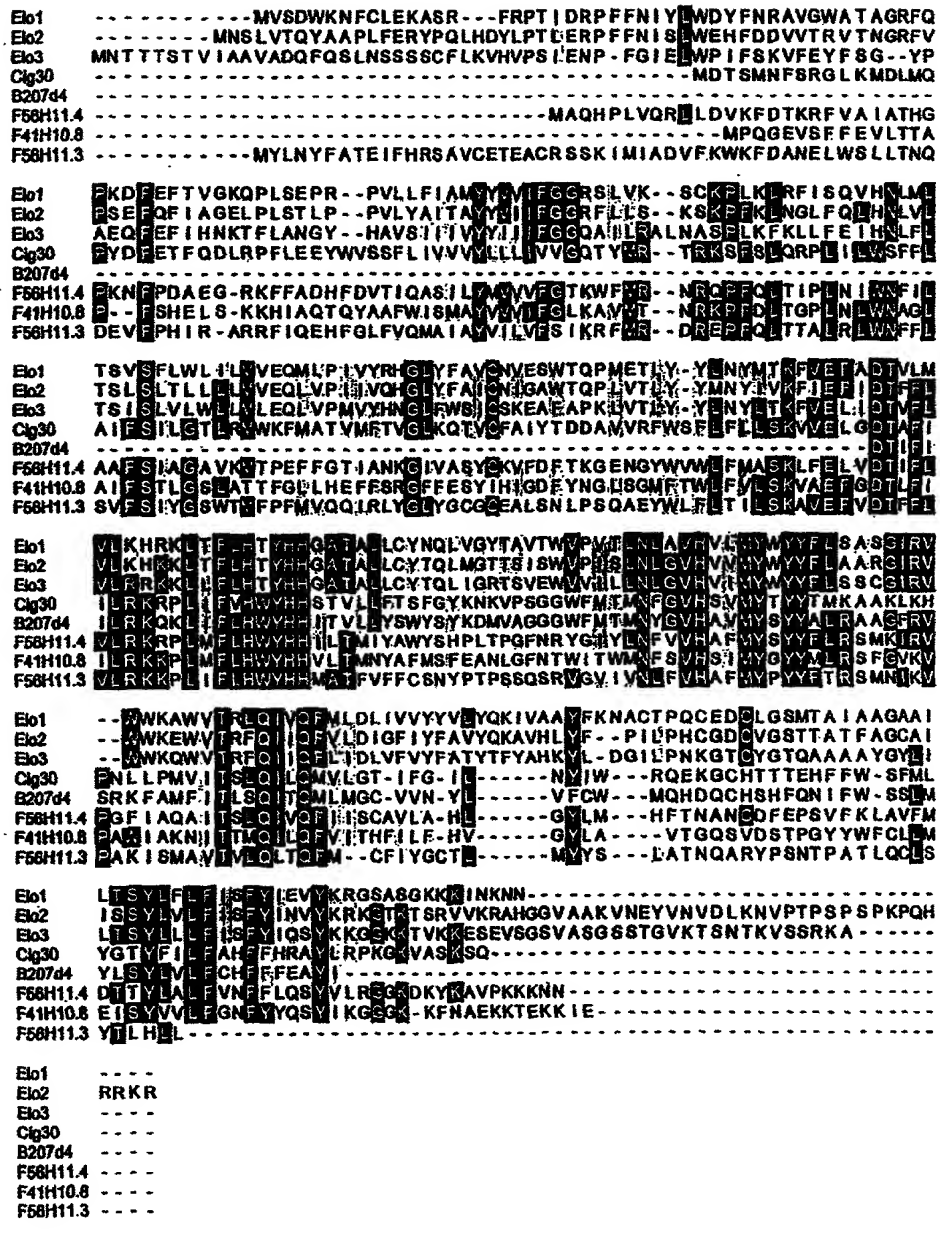
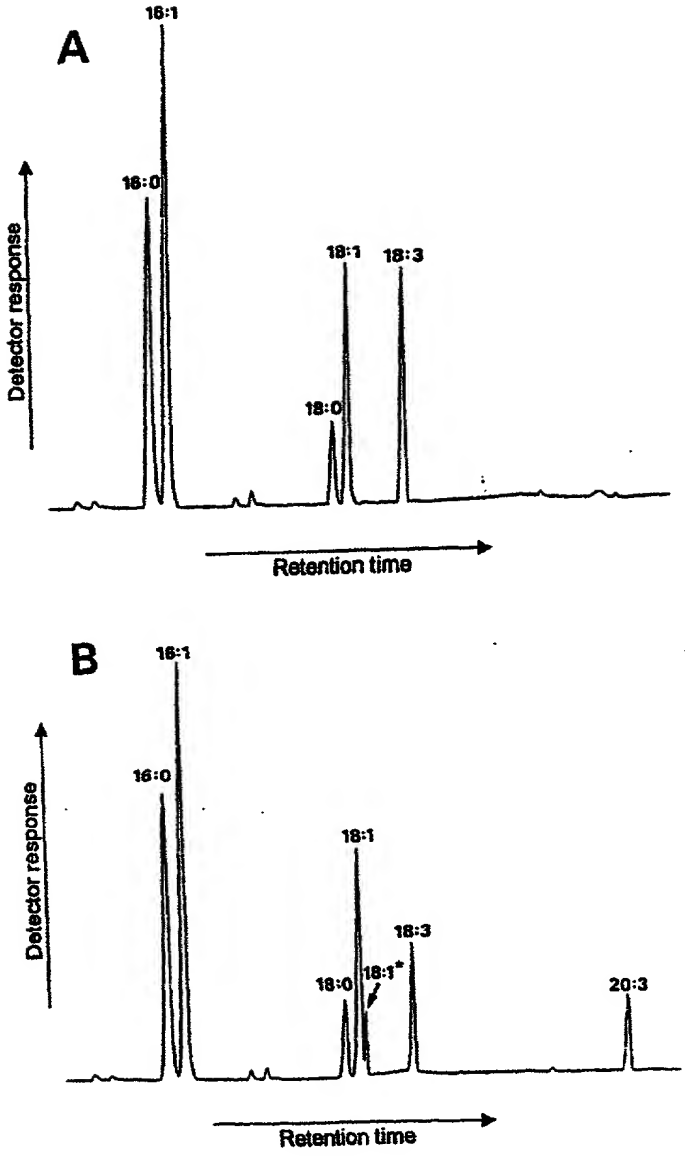


FIG. 11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Detector response
Retention time

SOLE DECLARATION FOR PATENT APPLICATION

As the below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I believe I am the original, first and sole inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled "Polysaturated fatty acid(PUFA) élongase from Caenorhabitis elegans" the specification of which

☐ is attached hereto.

☐ was filed on ____ as Application Serial Number ____ and was amended on ____ (if applicable).

■ was filed under the Patent Cooperation Treaty (PCT) and accorded International (PCT) Application No. PCT/GB00/01035, filed 20 March 2000 and amended on (if any).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I hereby acknowledge the duty to disclose information which is material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56(a).

Prior Foreign Application(s)

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application(s) for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Country	Application No.	Date of Filing (day month year)	Date of Issue (day month year)	Priority Claimed Under 35 U.S.C. §119
Great Britain	9906307.5	18 March 1999		Yes
Great Britain	0003869.5	18 February 2000		Yes

Prior United States Provisional Application(s)

I hereby claim priority benefits under Title 35, United States Code, §119(e)(1) of any U.S. provisional application listed below:

U.S. Provisional Application No.	Date of Filing (day month year)	Priority Claimed Under 35 U.S.C. §119(e)(1)

Prior United States Application(s)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Date of Filing (Day, Month, Year)	Status — Patented, Pending, Abandoned

Power of Attorney

And I hereby appoint, both jointly and severally, as my attorneys with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected herewith the following attorneys and agents, their registration numbers being listed after their names:

LTHERR, Robert F 31,810 BANNER, Donald W. 17,037

FORM PTO-1595 (8-83)

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JACKSON, Thomas H.	29,808	PATHAK, Ajay S.	38,266
KAGAN, Sarah A.	32,141	PAYNE, Stephen S.	35,316
KATZ, Robert S.	36,402	PETERSON, Thomas L.	30,969
KLEIN, William J.	43,719	POTENZA, Joseph M.	28,175
KRAUSE, Joseph P.	32,578	PRATT, Thomas K.	37,210
LINEK, Ernest V.	29,822	RENK, Christopher J.	33,761
MALONE, Dale A	32,155	RESIS, Robert H.	32,168
MANNAVA, Ashok K.	45,301	RIVARD, Paul M.	43,446
MAPLE, Marie-Claire B.	37,588	ROBINSON, Douglas W.	32,751
MAY, Steven A.	44,912	SCHAD, Steven P.	32,550
McDERMOTT, Peter D.	29,417	SHIPLEY, Charles W.	28,042
McKEE, Christopher L.	32,384	SKERPON, Joseph M.	29,864
McKIE, Edward F.	17,335	STOCKLEY, D. J.	34,257
MEDLOCK, Nina L	29,673	VAN ES, J. Pieter	37,746
MEECE, Timothy C.	38,553	WITCOFF, Sheldon W.	17,399
MEEKER, Frederic M.	35,282	WOLFF, Kevin A.	42,233
MILLER, Charles L.	43 805	WOLFFE, Franklin D.	19,724
MITRIUS, Janice V.	43,808	WOLFFE, Susan A	33,568
MORENO, Christopher P.	38,566	WRIGHT, Bradley C.	38,061
NELSON, Jon O.	24,566		

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Washington, D.C. 20001-4597 Fax: (202) 508-9299

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature _____ Date 3rd Sept 2001

Full Name of Sole Inventor NAPIER Johnathan A.
Family Name First Given Name Second Given Name

Residence Great Britain Citizenship British

Post Office Address IACR-Long Ashton Research Station, Dept. of Agriculture Sciences, University of Bristol, Long Ashton, Bristol BS41 9AF

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<110> Napier, Johnathan A.

<120> Polyunsaturated Fatty Acid (PUFA) Elongase from Caenorhabditis elegans

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<210> 7
<211> 876
<212> DNA
<213> C. elegans

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ccgcagtatt ggacgatttt attccagaaa tattggtatc attcgatcac aatatcagtt 180
ctttatttca ttttaattaa ggtgattcaa aagtttatgg agaatcgaaa accattcact 240
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ttgcggttct ctattgatcc tctacgatca ctatatgctg aaggattcta caaaactctg 360
tgctattcgt gtaatccaac tgatgtggct gcattttgga gctttgcatt cgctctttcc 420
aagattgttg aacttggaga cactatgttc attattttga gaaaacggcc attgatcttt 480
ttacactact atcatcatgc agcagtgtta atctacactg tccattctgg tgccgagcat 540
actgcagctg gtcgtttcta catcctaatg aactacttcg cacattctct catgtatact 600
tactacacag tttctgccat gggatacaga ttaccgaaat gggtatcaat gactgtcaca 660
actgttcaaa caactcaaat gttagctgga gtcggaataa cttggatggt gtacaaagtg 720
aaaactgaat acaagcttcc ttgtcaacaa tccgtagcca atttgtatct cgcattcgtc 780
atctatgtca catttgccat tcttttcatt caattcttcg tcaaggcata cattatcaag 840
tcgtcgaaga agtcgaaatc ggtgaagaac gaataa 876

<210> 8
<211> 1308
<212> DNA
<213> C. elegans

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tcaattacag catctgtcgt gtatgtagcc gtcattttta caggaaagaa ggtggttctc 180
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cgaaaccgaa aatcacttaa tagttctcaa atgtttcaga ttatggaaaa gtacaagccc 300
ttccaactgg acacaccact cttcgtctgg aattcatttt tagccatttt ctcaattctc 360
gggttcctcc gaatgacacc tgaatttgta tggagttggt cagcagaagg aaactcattc 420
aaatattcaa tttgtcattc atcttatgct caaggagtca ctggtttctg gactgaacaa 480
ttcgcaatga gcaaactttt cgagctcatc gacacaatct tcatcgttct tcgtaaacgt 540
ccactcatct tccttcactg gtatcatcat gtaactgtta tgatctacac atggcacgcg 600
tacaaggatc acactgcatc aggacggtgg ttcatttgga tgaattatgg agttcatgct 660
cttatgtatt cctactatgc tcttcgttct ctgaaattcc gtcttccaaa acaaatggca 720

atggttgtta ctactctcca acttgctcaa atggttatgg gagtaatcat cggagtcact 780
gtctaccgta tcaagtcatc gggtgaatac tgccaacaga catgggacaa tttgggatta 840
tgctttggag tttatttcac atatttcctt cttttcgcca acttcttcta ccatgcatat 900
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gttttaattt acctgagaaa aaaggtttca agaaaatcga aaaatcggca atgttcagaa 1020
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aaaacatatg aacttattct tccaagaaga aaaatgacca caattttaac ttttctattt 1140
ggaaaaaatc gaatttttc gaaatatcag aaaaatcgaa aaaacatttc gattcctgtt 1200
gatttcgaaa ttctggagcc aaaagaagat atcaatgcta acatcgctga gccatccatc 1260
acaacgaggt ccgccgccgc acgaagaaaa gttcaaaaag ctgattag 1308

<210> 9
<211> 825
<212> DNA
<213> C. elegans

<400> 9
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attggttatc tcgtcaccat ctacttcggg caaaaattaa tggctcacag aaaaccattc 180
gatctccaaa atacacttgc tctctggaac ttcgggtttt cactgttctc gggaatcgcc 240
gcctataagc ttattccaga actattcgga gttttcatga aggacgggtt tgtcgcttcc 300
tactgtcaaa acgagaacta ctacaccgat gcatcaactg gattctgggg ctgggccttt 360
gtgatgtcga aagctccaga actaggggat actatgttct tggtccttcg taaaaaacca 420
gttatcttca tgcactggta tcatcatgcc ctcacatttg tctacgcagt agtcacatac 480
tctgagcatc aggcatgggc tcgttggtct ttggctctca accttgccgt ccacactgtt 540
atgtatttct acttcgccgt tcgcgccttg aacatccaaa ctccacgccc agtggcaaag 600
ttcatcacta ctattcaaat tgtccaattt gtcatctcat gctacatttt tgggcatttg 660
gtattcatta agtctgctga ttctgttcct ggttgcgctg ttagctggaa tgtgctatcg 720
atcggaggac tcatgtacat cagttatttg ttccttttg ccaagttctt ctacaaggcc 780
tacattcaaa aacgctcacc aaccaaaacc agcaagcagg agtag 825

<210> 10
<211> 861
<212> DNA
<213> C. elegans

<400> 10
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cttgccgtcg gatatattgc cactattttt ggactgaaat attatatgaa agaccgaaaa 180
gccttcgatc tcagtactcc attaaacatt tggaatggta ttctttcgac attcagctta 240
ttgggattct tattcacttt tcctactttg ttatcagtta tcagaaagga tggatttagt 300
cacacctatt cccatgtctc tgagctttac actgacagta cctctggata ttggatcttc 360
ctttgggtta tctcaaagat tccggaactt ttggatacag tattcattgt tcttcgcaag 420
agaccactta ttttcatgca ctggtaccat cacgcattga ccggttacta tgctcttgtc 480
tgctaccatg aggatgctgt ccatatggtt tgggttgtat ggatgaatta tattattcat 540
gcattcatgt atggatacta tcttctgaaa tctctgaaag ttccaattcc accatcagtt 600
gctcaagcaa tcaccacatc tcaaatggtt caattcgcag ttgccatttt cgcacaagtt 660
catgtttcct ataaacacta tgttgaggga gttgaaggat tagcctactc gttcagagga 720
acagctatcg gatttttcat gcttactacc tacttctatc tatggattca attctacaaa 780
gagcactatc ttaagaatgg aggcaaaaag tacaatttgg caaaggatca ggcaaaaact 840
caaacaaaga aggctaacta a 861

<210> 11
<211> 825
<212> DNA
<213> C. elegans

<400> 11
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gttgtcgtta tttttgggct caaggctgtc atgacaaacc gaaaaccatt tgatctcacg 180
ggaccactga atctctggaa tgcgggtctt gctatttttct caactctcgg atcacttgcc 240
actacatttg gacttctcca cgagttcttc agccgtggat ttttcgaatc ttacattcac 300
atcggagact tttataatgg actttctgga atgttcacat ggcttttcgt tctctcaaaa 360
gttgctgaat tcggagatac actttttatt attcttcgta aaaagccatt gatgttcctt 420
cattggtatc atcatgtgct tacaatgaat tatgctttta tgtcatttga agctaatttg 480
ggatttaata cttggattac atggatgaat ttctcagttc actcaattat gtatggatat 540
tatatgcttc gttcttttgg tgtcaaggtt ccagcatgga ttgccaagaa tattacaaca 600
atgcaaattc ttcaattcgt tattactcat ttcattcttt tccacgttgg atatttggca 660
gttactggac aatctgttga ctcaactcca ggatattatt ggttctgcct tctcatggaa 720
atctcttatg tcgttctgtt cggaaacttc tactatcaat catacatcaa gggaggtggc 780
aagaagttta atgcagagaa gaagactgaa aagaaaattg aataa 825

<210> 12
<211> 846
<212> DNA
<213> C. elegans

<400> 12
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gaattgtgga gtcttttaac gaatcaggat gaagttttcc cgcatattag agcacggcga 180
ttcattcaag aacattttgg tctattcgtc cagatggcaa ttgcatatgt cattttggtg 240
ttctcaatca aaaggttcat gagggatcgt gaaccatttc aactcaccac agctcttcgt 300
ctctggaact tcttcctctc cgtcttctca atttatggtt cctggacaat gtttccattt 360
atggttcaac aaataagact ttatggtctc tacggatgtg gatgcgaagc actttcaaac 420
cttccgagtc aagcagaata ttggcttttc ctgacgatct tgtccaaagc tgtggagttt 480
gttgatacat ttttcttggt tctccggaaa aaaccactca tcttcctaca ctggtatcat 540
catatggcaa catttgtctt cttctgcagt aattacccga ctccatcgtc acaatcacgc 600
gtcggagtta tcgtcaacct gttcgtgcat gccttcatgt acccatacta tttcacccga 660
tcaatgaaca tcaaagttcc tgcgaaaatt tcaatggctg ttacagttct tcaattgact 720
caattcatgt gctttatcta tggatgtact ctcatgtact actcgttggc cactaatcag 780
gcacgatacc cctcaaatac acctgcgaca ctccaatgtt tgtcctacac tctacatttg 840
ctttga 846

<210> 13
<211> 866
<212> DNA
<213> C. elegans

<400> 13
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gctgatcact ttgatgttac tattcaggct tcaatcctgt acatggtcgt tgtgttcgga 180
acaaaatggt tcatgcgtaa tcgtcaacca ttccaattga ctattccact caacatctgg 240
aatttcatcc tcgccgcatt ttccatcgca ggagctgtca aaatgacccc agagttcttt 300
ggaaccattg ccaacaaagg aattgtcgat cctactgcaa agtgtttgat ttcacgaaag 360
gagagaatgg atactgggtg tggctcttca tggcttccaa acttttcgaa cttgttgaca 420
ccatcttctt ggttctccgt aaacgtccac tcatgttcct tcactggtat caccatattc 480
tcaccatgat ctacgcctgg tactctcatc cattgacccc aggattcaac agatacggaa 540
tttatcttaa ctttgtcgtc cacgccttca tgtactctta ctacttcctt cgctcgatga 600
agattcgcgt gccaggattc atcgcccaag ctatcacatc tcttcaaatc gttcaattca 660
tcatctcttg cgccgttctt gctcatcttg gttatctcat gcacttcacc aatgccaact 720
gtgatttcga gccatcagta ttcaagctcg cagttttcat ggacacaaca tacttggctc 780
ttttcgtcaa cttcttcctc caatcatatg ttctccgcgg aggaaaagac aagtacaagg 840
cagtgccaaa gaagaagaac aactaa 866

<210> 14
<211> 801
<212> DNA
<213> C. elegans

<400> 14
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ctgcttatat tattgcgaca aatttattac agagatatat ggagtcacgg aaacctaaaa 180
cttttactag catggaacgg ttttttggca gtgttcagta ttatgggtac atggagattt 240
ggaatcgaat tctacgatgc tgttttcaga agaggcttca tcgattcgat ctgcctggct 300
gtaaatccac gttcaccgtc cgcattctgg gcatgcatgt tcgctctatc gaaaatcgcc 360
gagtttgggg acacgatgtt cttggtgctg aggaaacggc cggttatatt ccttcactgg 420
tatcatcacg ctgttgttct gatcctttct tggcatgctg caatcgaact cacagctcca 480
ggacgctggt ttatttttat gaactatttg gtgcattcaa taatgtatac atactacgca 540
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actcttcaaa tgctcattgg tgtcagcatt tcttgcattg tgctttattt gaagcttaat 660
ggagagatgt gccaacaatc ctacgacaat ctggcgttga gcttcggaat ctacgcctca 720
ttcctggtgc tattctccag tttcttcaac aatgcatatt tggtaaaaaa ggacaagaaa 780
cccgatgtga agaaggatta a 801

<210> 15
<211> 291
<212> PRT
<213> C. elegans

<400> 15
Met Glu Leu Ala Glu Phe Trp Asn Asp Leu Asn Thr Phe Thr Ile Tyr
1 5 10 15

Gly Pro Asn His Thr Asp Met Thr Thr Lys Tyr Lys Tyr Ser Tyr His
20 25 30

Phe Pro Gly Glu Gln Val Ala Asp Pro Gln Tyr Trp Thr Ile Leu Phe
35 40 45

Gln Lys Tyr Trp Tyr His Ser Ile Thr Ile Ser Val Leu Tyr Phe Ile
50 55 60

Leu Ile Lys Val Ile Gln Lys Phe Met Glu Asn Arg Lys Pro Phe Thr
65 70 75 80

Leu Lys Tyr Pro Leu Ile Leu Trp Asn Gly Ala Leu Ala Ala Phe Ser
85 90 95

Ile Ile Ala Thr Leu Arg Phe Ser Ile Asp Pro Leu Arg Ser Leu Tyr
100 105 110

Ala Glu Gly Phe Tyr Lys Thr Leu Cys Tyr Ser Cys Asn Pro Thr Asp
115 120 125

Val Ala Ala Phe Trp Ser Phe Ala Phe Ala Leu Ser Lys Ile Val Glu
130 135 140

Leu Gly Asp Thr Met Phe Ile Ile Leu Arg Lys Arg Pro Leu Ile Phe
145 150 155 160

Leu His Tyr Tyr His His Ala Ala Val Leu Ile Tyr Thr Val His Ser
165 170 175

Gly Ala Glu His Thr Ala Ala Gly Arg Phe Tyr Ile Leu Met Asn Tyr
180 185 190

Phe Ala His Ser Leu Met Tyr Thr Tyr Tyr Thr Val Ser Ala Met Gly
195 200 205

Tyr Arg Leu Pro Lys Trp Val Ser Met Thr Val Thr Thr Val Gln Thr
210 215 220

Thr Gln Met Leu Ala Gly Val Gly Ile Thr Trp Met Val Tyr Lys Val
225 230 235 240

Lys Thr Glu Tyr Lys Leu Pro Cys Gln Gln Ser Val Ala Asn Leu Tyr
245 250 255

Leu Ala Phe Val Ile Tyr Val Thr Phe Ala Ile Leu Phe Ile Gln Phe
260 265 270

Phe Val Lys Ala Tyr Ile Ile Lys Ser Ser Lys Lys Ser Lys Ser Val
275 280 285

Lys Asn Glu
290

<210> 16
<211> 435
<212> PRT
<213> C. elegans

<400> 16
Met Ala Lys Tyr Asp Tyr Asn Pro Lys Tyr Gly Leu Glu Asn Tyr Ser
1 5 10 15

Ile Phe Leu Pro Phe Glu Thr Ser Phe Asp Ala Phe Arg Ser Thr Thr
20 25 30

Trp Met Gln Asn His Trp Tyr Gln Ser Ile Thr Ala Ser Val Val Tyr
35 40 45

Val Ala Val Ile Phe Thr Gly Lys Lys Val Val Leu Ile Tyr Lys Lys
50 55 60

Ser Arg Val Ile Thr Phe Glu Ser Ser Leu Gln Asn Ala Ile Lys Asn
65 70 75 80

Arg Asn Arg Lys Ser Leu Asn Ser Ser Gln Met Phe Gln Ile Met Glu
85 90 95

Lys Tyr Lys Pro Phe Gln Leu Asp Thr Pro Leu Phe Val Trp Asn Ser
100 105 110

Phe Leu Ala Ile Phe Ser Ile Leu Gly Phe Leu Arg Met Thr Pro Glu
115 120 125

Phe Val Trp Ser Trp Ser Ala Glu Gly Asn Ser Phe Lys Tyr Ser Ile
130 135 140

Cys His Ser Ser Tyr Ala Gln Gly Val Thr Gly Phe Trp Thr Glu Gln
145 150 155 160

Phe Ala Met Ser Lys Leu Phe Glu Leu Ile Asp Thr Ile Phe Ile Val
165 170 175

Leu Arg Lys Arg Pro Leu Ile Phe Leu His Trp Tyr His His Val Thr
180 185 190

Val Met Ile Tyr Thr Trp His Ala Tyr Lys Asp His Thr Ala Ser Gly
195 200 205

Arg Trp Phe Ile Trp Met Asn Tyr Gly Val His Ala Leu Met Tyr Ser
210 215 220

Tyr Tyr Ala Leu Arg Ser Leu Lys Phe Arg Leu Pro Lys Gln Met Ala
225 230 235 240

Met Val Val Thr Thr Leu Gln Leu Ala Gln Met Val Met Gly Val Ile
245 250 255

Ile Gly Val Thr Val Tyr Arg Ile Lys Ser Ser Gly Glu Tyr Cys Gln
260 265 270

Gln Thr Trp Asp Asn Leu Gly Leu Cys Phe Gly Val Tyr Phe Thr Tyr
275 280 285

Phe Leu Leu Phe Ala Asn Phe Phe Tyr His Ala Tyr Val Lys Lys Asn
290 295 300

Asn Arg Thr Val Asn Tyr Glu Asn Asn Ser Lys Asn Phe Pro Asp Leu
305 310 315 320

Val Leu Ile Tyr Leu Arg Lys Lys Val Ser Arg Lys Ser Lys Asn Arg
325 330 335

Gln Cys Ser Glu Asn Asn Tyr Lys Ile Gln Phe Ser Ser Asn Phe Val
340 345 350

Asn Val Asp Gly Lys Lys His Lys Lys Thr Tyr Glu Leu Ile Leu Pro
355 360 365

Arg Arg Lys Met Thr Thr Ile Leu Thr Phe Leu Phe Gly Lys Asn Arg
370 375 380

Ile Phe Ser Lys Tyr Gln Lys Asn Arg Lys Asn Ile Ser Ile Pro Val
385 390 395 400

Asp Phe Glu Ile Leu Glu Pro Lys Glu Asp Ile Asn Ala Asn Ile Ala
405 410 415

Glu Pro Ser Ile Thr Thr Arg Ser Ala Ala Ala Arg Arg Lys Val Gln
420 425 430

Lys Ala Asp
435

<210> 17
<211> 274
<212> PRT
<213> C. elegans

<400> 17
Met Ala Ala Ala Gln Thr Ser Pro Ala Ala Thr Leu Val Asp Val Leu
1 5 10 15

Thr Lys Pro Trp Ser Leu Asp Gln Thr Asp Ser Tyr Met Ser Thr Phe
20 25 30

Val Pro Leu Ser Tyr Lys Ile Met Ile Gly Tyr Leu Val Thr Ile Tyr
35 40 45

Phe Gly Gln Lys Leu Met Ala His Arg Lys Pro Phe Asp Leu Gln Asn
50 55 60

Thr Leu Ala Leu Trp Asn Phe Gly Phe Ser Leu Phe Ser Gly Ile Ala
65 70 75 80

Ala Tyr Lys Leu Ile Pro Glu Leu Phe Gly Val Phe Met Lys Asp Gly
85 90 95

Phe Val Ala Ser Tyr Cys Gln Asn Glu Asn Tyr Tyr Thr Asp Ala Ser
100 105 110

Thr Gly Phe Trp Gly Trp Ala Phe Val Met Ser Lys Ala Pro Glu Leu
115 120 125

Gly Asp Thr Met Phe Leu Val Leu Arg Lys Lys Pro Val Ile Phe Met
130 135 140

His Trp Tyr His His Ala Leu Thr Phe Val Tyr Ala Val Val Thr Tyr
145 150 155 160

Ser Glu His Gln Ala Trp Ala Arg Trp Ser Leu Ala Leu Asn Leu Ala
165 170 175

Val His Thr Val Met Tyr Phe Tyr Phe Ala Val Arg Ala Leu Asn Ile
180 185 190

Gln Thr Pro Arg Pro Val Ala Lys Phe Ile Thr Thr Ile Gln Ile Val
195 200 205

Gln Phe Val Ile Ser Cys Tyr Ile Phe Gly His Leu Val Phe Ile Lys
210 215 220

Ser Ala Asp Ser Val Pro Gly Cys Ala Val Ser Trp Asn Val Leu Ser
225 230 235 240

Ile Gly Gly Leu Met Tyr Ile Ser Tyr Leu Phe Leu Phe Ala Lys Phe
245 250 255

Phe Tyr Lys Ala Tyr Ile Gln Lys Arg Ser Pro Thr Lys Thr Ser Lys
260 265 270

Gln Glu

<210> 18
<211> 286
<212> PRT
<213> C. elegans

<400> 18
Met Ser Ser Asp Asp Arg Gly Thr Arg Thr Phe Lys Met Met Asp Gln
1 5 10 15

Ile Leu Gly Thr Asn Phe Thr Tyr Glu Gly Ala Lys Glu Val Ala Arg
20 25 30

Gly Leu Glu Gly Phe Ser Ala Lys Leu Ala Val Gly Tyr Ile Ala Thr
35 40 45

Ile Phe Gly Leu Lys Tyr Tyr Met Lys Asp Arg Lys Ala Phe Asp Leu
50 55 60

Ser Thr Pro Leu Asn Ile Trp Asn Gly Ile Leu Ser Thr Phe Ser Leu

65 70 75 80

Leu Gly Phe Leu Phe Thr Phe Pro Thr Leu Leu Ser Val Ile Arg Lys
85 90 95

Asp Gly Phe Ser His Thr Tyr Ser His Val Ser Glu Leu Tyr Thr Asp
100 105 110

Ser Thr Ser Gly Tyr Trp Ile Phe Leu Trp Val Ile Ser Lys Ile Pro
115 120 125

Glu Leu Leu Asp Thr Val Phe Ile Val Leu Arg Lys Arg Pro Leu Ile
130 135 140

Phe Met His Trp Tyr His His Ala Leu Thr Gly Tyr Tyr Ala Leu Val
145 150 155 160

Cys Tyr His Glu Asp Ala Val His Met Val Trp Val Val Trp Met Asn
165 170 175

Tyr Ile Ile His Ala Phe Met Tyr Gly Tyr Tyr Leu Leu Lys Ser Leu
180 185 190

Lys Val Pro Ile Pro Pro Ser Val Ala Gln Ala Ile Thr Thr Ser Gln
195 200 205

Met Val Gln Phe Ala Val Ala Ile Phe Ala Gln Val His Val Ser Tyr
210 215 220

Lys His Tyr Val Glu Gly Val Glu Gly Leu Ala Tyr Ser Phe Arg Gly
225 230 235 240

Thr Ala Ile Gly Phe Phe Met Leu Thr Thr Tyr Phe Tyr Leu Trp Ile
245 250 255

Gln Phe Tyr Lys Glu His Tyr Leu Lys Asn Gly Gly Lys Lys Tyr Asn
260 265 270

Leu Ala Lys Asp Gln Ala Lys Thr Gln Thr Lys Lys Ala Asn
275 280 285

<210> 19
<211> 274
<212> PRT
<213> C. elegans

<400> 19
Met Pro Gln Gly Glu Val Ser Phe Phe Glu Val Leu Thr Thr Ala Pro
1 5 10 15

Phe Ser His Glu Leu Ser Lys Lys His Ile Ala Gln Thr Gln Tyr Ala
20 25 30

Ala Phe Trp Ile Ser Met Ala Tyr Val Val Val Ile Phe Gly Leu Lys
35 40 45

Ala Val Met Thr Asn Arg Lys Pro Phe Asp Leu Thr Gly Pro Leu Asn
50 55 60

Leu Trp Asn Ala Gly Leu Ala Ile Phe Ser Thr Leu Gly Ser Leu Ala
65 70 75 80

Thr Thr Phe Gly Leu Leu His Glu Phe Phe Ser Arg Gly Phe Phe Glu
85 90 95

Ser Tyr Ile His Ile Gly Asp Phe Tyr Asn Gly Leu Ser Gly Met Phe
100 105 110

Thr Trp Leu Phe Val Leu Ser Lys Val Ala Glu Phe Gly Asp Thr Leu
115 120 125

Phe Ile Ile Leu Arg Lys Lys Pro Leu Met Phe Leu His Trp Tyr His
130 135 140

His Val Leu Thr Met Asn Tyr Ala Phe Met Ser Phe Glu Ala Asn Leu
145 150 155 160

Gly Phe Asn Thr Trp Ile Thr Trp Met Asn Phe Ser Val His Ser Ile
165 170 175

Met Tyr Gly Tyr Tyr Met Leu Arg Ser Phe Gly Val Lys Val Pro Ala
180 185 190

Trp Ile Ala Lys Asn Ile Thr Thr Met Gln Ile Leu Gln Phe Val Ile
195 200 205

Thr His Phe Ile Leu Phe His Val Gly Tyr Leu Ala Val Thr Gly Gln
210 215 220

Ser Val Asp Ser Thr Pro Gly Tyr Tyr Trp Phe Cys Leu Leu Met Glu
225 230 235 240

Ile Ser Tyr Val Val Leu Phe Gly Asn Phe Tyr Tyr Gln Ser Tyr Ile
245 250 255

Lys Gly Gly Gly Lys Lys Phe Asn Ala Glu Lys Lys Thr Glu Lys Lys
260 265 270

Ile Glu

<210> 20
<211> 281
<212> PRT
<213> C. elegans

<400> 20

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Met Tyr Leu Asn Tyr Phe Ala Thr Glu Ile Phe His Arg Ser Ala Val
 1               5                  10                  15

Cys Glu Thr Glu Ala Cys Arg Ser Ser Lys Ile Met Ile Ala Asp Val
            20                  25                  30

Phe Lys Trp Lys Phe Asp Ala Asn Glu Leu Trp Ser Leu Leu Thr Asn
        35                  40                  45

Gln Asp Glu Val Phe Pro His Ile Arg Ala Arg Arg Phe Ile Gln Glu
    50                  55                  60

His Phe Gly Leu Phe Val Gln Met Ala Ile Ala Tyr Val Ile Leu Val
65                  70                  75                  80

Phe Ser Ile Lys Arg Phe Met Arg Asp Arg Glu Pro Phe Gln Leu Thr
                85                  90                  95

Thr Ala Leu Arg Leu Trp Asn Phe Phe Leu Ser Val Phe Ser Ile Tyr
            100                 105                 110

Gly Ser Trp Thr Met Phe Pro Phe Met Val Gln Gln Ile Arg Leu Tyr
        115                 120                 125

Gly Leu Tyr Gly Cys Gly Cys Glu Ala Leu Ser Asn Leu Pro Ser Gln
    130                 135                 140

Ala Glu Tyr Trp Leu Phe Leu Thr Ile Leu Ser Lys Ala Val Glu Phe
145                 150                 155                 160

Val Asp Thr Phe Phe Leu Val Leu Arg Lys Lys Pro Leu Ile Phe Leu
                165                 170                 175

His Trp Tyr His His Met Ala Thr Phe Val Phe Phe Cys Ser Asn Tyr
            180                 185                 190

Pro Thr Pro Ser Ser Gln Ser Arg Val Gly Val Ile Val Asn Leu Phe
        195                 200                 205

Val His Ala Phe Met Tyr Pro Tyr Tyr Phe Thr Arg Ser Met Asn Ile
    210                 215                 220

Lys Val Pro Ala Lys Ile Ser Met Ala Val Thr Val Leu Gln Leu Thr
225                 230                 235                 240

Gln Phe Met Cys Phe Ile Tyr Gly Cys Thr Leu Met Tyr Tyr Ser Leu
                245                 250                 255

Ala Thr Asn Gln Ala Arg Tyr Pro Ser Asn Thr Pro Ala Thr Leu Gln
            260                 265                 270

Cys Leu Ser Tyr Thr Leu His Leu Leu
        275                 280
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<210> 21
<211> 288
<212> PRT
<213> C. elegans

<400> 21

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Met Ala Gln His Pro Leu Val Gln Arg Leu Leu Asp Val Lys Phe Asp
  1               5                  10                  15

Thr Lys Arg Phe Val Ala Ile Ala Thr His Gly Pro Lys Asn Phe Pro
             20                  25                  30

Asp Ala Glu Gly Arg Lys Phe Phe Ala Asp His Phe Asp Val Thr Ile
         35                  40                  45

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Asn Phe Ile Leu Ala Ala Phe Ser Ile Ala Gly Ala Val Lys Met Thr
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Pro Glu Phe Phe Gly Thr Ile Ala Asn Lys Gly Ile Val Ala Ser Tyr
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Cys Lys Val Phe Asp Phe Thr Lys Gly Glu Asn Gly Tyr Trp Val Trp
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Val Leu Arg Lys Arg Pro Leu Met Phe Leu His Trp Tyr His His Ile
145                 150                 155                 160

Leu Thr Met Ile Tyr Ala Trp Tyr Ser His Pro Leu Thr Pro Gly Phe
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Asn Arg Tyr Gly Ile Tyr Leu Asn Phe Val Val His Ala Phe Met Tyr
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Ser Tyr Tyr Phe Leu Arg Ser Met Lys Ile Arg Val Pro Gly Phe Ile
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Ala Gln Ala Ile Thr Ser Leu Gln Ile Val Gln Phe Ile Ile Ser Cys
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Cys Asp Phe Glu Pro Ser Val Phe Lys Leu Ala Val Phe Met Asp Thr
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Thr Trp Arg Phe Gly Ile Glu Phe Tyr Asp Ala Val Phe Arg Xaa Gly
                 85                  90                  95

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Tyr His His Ala Val Val Leu Ile Leu Ser Trp His Ala Ala Ile Glu
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Leu Thr Ala Pro Gly Arg Trp Phe Ile Phe Met Asn Tyr Leu Val His
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Pro Lys Ile Val Ser Met Thr Val Thr Phe Leu Gln Thr Leu Gln Met
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Leu Ile Gly Val Ser Ile Ser Cys Ile Val Leu Tyr Leu Lys Leu Asn
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Gly Glu Met Cys Gln Gln Ser Tyr Asp Asn Leu Ala Leu Ser Phe Gly
225                 230                 235                 240

Ile Tyr Ala Ser Phe Leu Val Leu Ser Ser Phe Phe Asn Asn Ala Tyr
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09/936845

JC16 Rec'd PCT/PTO SEP 1 8 2001

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<130> 76/7

<140> PCT/GB00/01035
<141> 2000-03-20

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<170> PatentIn Ver. 2.1

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Gln Lys Tyr Trp Tyr His Ser Ile Thr Ile Ser Val Leu Tyr Phe Ile
50 55 60

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Leu Lys Tyr Pro Leu Ile Leu Trp Asn Gly Ala Leu Ala Ala Phe Ser
                85                  90                  95

Ile Ile Ala Thr Leu Arg Phe Ser Ile Asp Pro Leu Arg Ser Leu Tyr
            100                 105                 110

Ala Glu Gly Phe Tyr Lys Thr Leu Cys Tyr Ser Cys Asn Pro Thr Asp
        115                 120                 125

Val Ala Ala Phe Trp Ser Phe Ala Phe Ala Leu Ser Lys Ile Val Glu
    130                 135                 140

Leu Gly Asp Thr Met Phe Ile Ile Leu Arg Lys Arg Pro Leu Ile Phe
145                 150                 155                 160

Leu His Tyr Tyr His His Ala Ala Val Leu Ile Tyr Thr Val His Ser
                165                 170                 175

Gly Ala Glu His Thr Ala Ala Gly Arg Phe Tyr Ile Leu Met Asn Tyr
            180                 185                 190

Phe Ala His Ser Leu Met Tyr Thr Tyr Tyr Thr Val Ser Ala Met Gly
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Tyr Arg Leu Pro Lys Trp Val Ser Met Thr Val Thr Thr Val Gln Thr
    210                 215                 220

Thr Gln Met Leu Ala Gly Val Gly Ile Thr Trp Met Val Tyr Lys Val
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Lys Thr Glu Tyr Lys Leu Pro Cys Gln Gln Ser Val Ala Asn Leu Tyr
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Leu Ala Phe Val Ile Tyr Val Thr Phe Ala Ile Leu Phe Ile Gln Phe
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     50                  55                  60

Ser Arg Val Ile Thr Phe Glu Ser Ser Leu Gln Asn Ala Ile Lys Asn
 65                  70                  75                  80

Arg Asn Arg Lys Ser Leu Asn Ser Ser Gln Met Phe Gln Ile Met Glu
                 85                  90                  95

Lys Tyr Lys Pro Phe Gln Leu Asp Thr Pro Leu Phe Val Trp Asn Ser
            100                 105                 110

Phe Leu Ala Ile Phe Ser Ile Leu Gly Phe Leu Arg Met Thr Pro Glu
        115                 120                 125

Phe Val Trp Ser Trp Ser Ala Glu Gly Asn Ser Phe Lys Tyr Ser Ile
    130                 135                 140

Cys His Ser Ser Tyr Ala Gln Gly Val Thr Gly Phe Trp Thr Glu Gln
145                 150                 155                 160

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Leu Arg Lys Arg Pro Leu Ile Phe Leu His Trp Tyr His His Val Thr
            180                 185                 190

Val Met Ile Tyr Thr Trp His Ala Tyr Lys Asp His Thr Ala Ser Gly
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Arg Trp Phe Ile Trp Met Asn Tyr Gly Val His Ala Leu Met Tyr Ser
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Tyr Tyr Ala Leu Arg Ser Leu Lys Phe Arg Leu Pro Lys Gln Met Ala
225                 230                 235                 240

Met Val Val Thr Thr Leu Gln Leu Ala Gln Met Val Met Gly Val Ile
                245                 250                 255

Ile Gly Val Thr Val Tyr Arg Ile Lys Ser Ser Gly Glu Tyr Cys Gln
            260                 265                 270

Gln Thr Trp Asp Asn Leu Gly Leu Cys Phe Gly Val Tyr Phe Thr Tyr
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Phe Leu Leu Phe Ala Asn Phe Phe Tyr His Ala Tyr Val Lys Lys Asn
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370 375 380

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Lys Ala Asp
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50 55 60

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Phe Val Ala Ser Tyr Cys Gln Asn Glu Asn Tyr Tyr Thr Asp Ala Ser
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Thr Gly Phe Trp Gly Trp Ala Phe Val Met Ser Lys Ala Pro Glu Leu
115 120 125

Gly Asp Thr Met Phe Leu Val Leu Arg Lys Lys Pro Val Ile Phe Met
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195 200 205

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210 215 220

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Gln Glu

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35 40 45

Ile Phe Gly Leu Lys Tyr Tyr Met Lys Asp Arg Lys Ala Phe Asp Leu
50 55 60

Ser Thr Pro Leu Asn Ile Trp Asn Gly Ile Leu Ser Thr Phe Ser Leu

```
 65                  70                  75                  80

Leu Gly Phe Leu Phe Thr Phe Pro Thr Leu Leu Ser Val Ile Arg Lys
                 85                  90                  95

Asp Gly Phe Ser His Thr Tyr Ser His Val Ser Glu Leu Tyr Thr Asp
            100                 105                 110

Ser Thr Ser Gly Tyr Trp Ile Phe Leu Trp Val Ile Ser Lys Ile Pro
        115                 120                 125

Glu Leu Leu Asp Thr Val Phe Ile Val Leu Arg Lys Arg Pro Leu Ile
    130                 135                 140

Phe Met His Trp Tyr His His Ala Leu Thr Gly Tyr Tyr Ala Leu Val
145                 150                 155                 160

Cys Tyr His Glu Asp Ala Val His Met Val Trp Val Val Trp Met Asn
                165                 170                 175

Tyr Ile Ile His Ala Phe Met Tyr Gly Tyr Tyr Leu Leu Lys Ser Leu
            180                 185                 190

Lys Val Pro Ile Pro Pro Ser Val Ala Gln Ala Ile Thr Thr Ser Gln
        195                 200                 205

Met Val Gln Phe Ala Val Ala Ile Phe Ala Gln Val His Val Ser Tyr
    210                 215                 220

Lys His Tyr Val Glu Gly Val Glu Gly Leu Ala Tyr Ser Phe Arg Gly
225                 230                 235                 240

Thr Ala Ile Gly Phe Phe Met Leu Thr Thr Tyr Phe Tyr Leu Trp Ile
                245                 250                 255

Gln Phe Tyr Lys Glu His Tyr Leu Lys Asn Gly Gly Lys Lys Tyr Asn
            260                 265                 270

Leu Ala Lys Asp Gln Ala Lys Thr Gln Thr Lys Lys Ala Asn
        275                 280                 285
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<210> 19
<211> 274
<212> PRT
<213> C. elegans

<400> 19

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Met Pro Gln Gly Glu Val Ser Phe Phe Glu Val Leu Thr Thr Ala Pro
  1               5                  10                  15

Phe Ser His Glu Leu Ser Lys Lys His Ile Ala Gln Thr Gln Tyr Ala
             20                  25                  30
```

```
Ala Phe Trp Ile Ser Met Ala Tyr Val Val Val Ile Phe Gly Leu Lys
          35                  40                  45

Ala Val Met Thr Asn Arg Lys Pro Phe Asp Leu Thr Gly Pro Leu Asn
      50                  55                  60

Leu Trp Asn Ala Gly Leu Ala Ile Phe Ser Thr Leu Gly Ser Leu Ala
 65                  70                  75                  80

Thr Thr Phe Gly Leu Leu His Glu Phe Phe Ser Arg Gly Phe Phe Glu
                 85                  90                  95

Ser Tyr Ile His Ile Gly Asp Phe Tyr Asn Gly Leu Ser Gly Met Phe
            100                 105                 110

Thr Trp Leu Phe Val Leu Ser Lys Val Ala Glu Phe Gly Asp Thr Leu
        115                 120                 125

Phe Ile Ile Leu Arg Lys Lys Pro Leu Met Phe Leu His Trp Tyr His
    130                 135                 140

His Val Leu Thr Met Asn Tyr Ala Phe Met Ser Phe Glu Ala Asn Leu
145                 150                 155                 160

Gly Phe Asn Thr Trp Ile Thr Trp Met Asn Phe Ser Val His Ser Ile
                165                 170                 175

Met Tyr Gly Tyr Tyr Met Leu Arg Ser Phe Gly Val Lys Val Pro Ala
            180                 185                 190

Trp Ile Ala Lys Asn Ile Thr Thr Met Gln Ile Leu Gln Phe Val Ile
        195                 200                 205

Thr His Phe Ile Leu Phe His Val Gly Tyr Leu Ala Val Thr Gly Gln
    210                 215                 220

Ser Val Asp Ser Thr Pro Gly Tyr Tyr Trp Phe Cys Leu Leu Met Glu
225                 230                 235                 240

Ile Ser Tyr Val Val Leu Phe Gly Asn Phe Tyr Tyr Gln Ser Tyr Ile
                245                 250                 255

Lys Gly Gly Gly Lys Lys Phe Asn Ala Glu Lys Lys Thr Glu Lys Lys
            260                 265                 270

Ile Glu
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<210> 20
<211> 281
<212> PRT
<213> C. elegans

<400> 20

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Met Tyr Leu Asn Tyr Phe Ala Thr Glu Ile Phe His Arg Ser Ala Val
 1               5                  10                  15

Cys Glu Thr Glu Ala Cys Arg Ser Ser Lys Ile Met Ile Ala Asp Val
            20                  25                  30

Phe Lys Trp Lys Phe Asp Ala Asn Glu Leu Trp Ser Leu Leu Thr Asn
        35                  40                  45

Gln Asp Glu Val Phe Pro His Ile Arg Ala Arg Arg Phe Ile Gln Glu
    50                  55                  60

His Phe Gly Leu Phe Val Gln Met Ala Ile Ala Tyr Val Ile Leu Val
65                  70                  75                  80

Phe Ser Ile Lys Arg Phe Met Arg Asp Arg Glu Pro Phe Gln Leu Thr
                85                  90                  95

Thr Ala Leu Arg Leu Trp Asn Phe Phe Leu Ser Val Phe Ser Ile Tyr
            100                 105                 110

Gly Ser Trp Thr Met Phe Pro Phe Met Val Gln Gln Ile Arg Leu Tyr
        115                 120                 125

Gly Leu Tyr Gly Cys Gly Cys Glu Ala Leu Ser Asn Leu Pro Ser Gln
    130                 135                 140

Ala Glu Tyr Trp Leu Phe Leu Thr Ile Leu Ser Lys Ala Val Glu Phe
145                 150                 155                 160

Val Asp Thr Phe Phe Leu Val Leu Arg Lys Lys Pro Leu Ile Phe Leu
                165                 170                 175

His Trp Tyr His His Met Ala Thr Phe Val Phe Phe Cys Ser Asn Tyr
            180                 185                 190

Pro Thr Pro Ser Ser Gln Ser Arg Val Gly Val Ile Val Asn Leu Phe
        195                 200                 205

Val His Ala Phe Met Tyr Pro Tyr Tyr Phe Thr Arg Ser Met Asn Ile
    210                 215                 220

Lys Val Pro Ala Lys Ile Ser Met Ala Val Thr Val Leu Gln Leu Thr
225                 230                 235                 240

Gln Phe Met Cys Phe Ile Tyr Gly Cys Thr Leu Met Tyr Tyr Ser Leu
                245                 250                 255

Ala Thr Asn Gln Ala Arg Tyr Pro Ser Asn Thr Pro Ala Thr Leu Gln
            260                 265                 270

Cys Leu Ser Tyr Thr Leu His Leu Leu
        275                 280
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<210> 21
<211> 288
<212> PRT
<213> C. elegans

<400> 21

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Met Ala Gln His Pro Leu Val Gln Arg Leu Leu Asp Val Lys Phe Asp
  1               5                  10                  15

Thr Lys Arg Phe Val Ala Ile Ala Thr His Gly Pro Lys Asn Phe Pro
             20                  25                  30

Asp Ala Glu Gly Arg Lys Phe Phe Ala Asp His Phe Asp Val Thr Ile
         35                  40                  45

Gln Ala Ser Ile Leu Tyr Met Val Val Val Phe Gly Thr Lys Trp Phe
     50                  55                  60

Met Arg Asn Arg Gln Pro Phe Gln Leu Thr Ile Pro Leu Asn Ile Trp
 65                  70                  75                  80

Asn Phe Ile Leu Ala Ala Phe Ser Ile Ala Gly Ala Val Lys Met Thr
                 85                  90                  95

Pro Glu Phe Phe Gly Thr Ile Ala Asn Lys Gly Ile Val Ala Ser Tyr
            100                 105                 110

Cys Lys Val Phe Asp Phe Thr Lys Gly Glu Asn Gly Tyr Trp Val Trp
        115                 120                 125

Leu Phe Met Ala Ser Lys Leu Phe Glu Leu Val Asp Thr Ile Phe Leu
    130                 135                 140

Val Leu Arg Lys Arg Pro Leu Met Phe Leu His Trp Tyr His His Ile
145                 150                 155                 160

Leu Thr Met Ile Tyr Ala Trp Tyr Ser His Pro Leu Thr Pro Gly Phe
                165                 170                 175

Asn Arg Tyr Gly Ile Tyr Leu Asn Phe Val Val His Ala Phe Met Tyr
            180                 185                 190

Ser Tyr Tyr Phe Leu Arg Ser Met Lys Ile Arg Val Pro Gly Phe Ile
        195                 200                 205

Ala Gln Ala Ile Thr Ser Leu Gln Ile Val Gln Phe Ile Ile Ser Cys
    210                 215                 220

Ala Val Leu Ala His Leu Gly Tyr Leu Met His Phe Thr Asn Ala Asn
225                 230                 235                 240

Cys Asp Phe Glu Pro Ser Val Phe Lys Leu Ala Val Phe Met Asp Thr
                245                 250                 255
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Thr Tyr Leu Ala Leu Phe Val Asn Phe Phe Leu Gln Ser Tyr Val Leu
260 265 270

Arg Gly Gly Lys Asp Lys Tyr Lys Ala Val Pro Lys Lys Lys Asn Asn
275 280 285

<210> 22
<211> 269
<212> PRT
<213> C. elegans

<400> 22
Met Ser Ala Glu Val Ser Glu Arg Phe Lys Val Trp Thr Gly Asn Asn
1 5 10 15

Glu Thr Ile Ile Tyr Ser Pro Phe Glu Tyr Asp Ser Thr Leu Leu Ile
20 25 30

Glu Ser Cys Arg Cys Thr Tyr Gln Leu Leu Ile Leu Leu Arg Gln Ile
35 40 45

Tyr Tyr Arg Asp Ile Trp Ser His Gly Asn Leu Lys Ala Cys Asp Xaa
50 55 60

Leu Leu Leu Ala Trp Asn Gly Phe Leu Ala Val Phe Ser Ile Met Gly
65 70 75 80

Thr Trp Arg Phe Gly Ile Glu Phe Tyr Asp Ala Val Phe Arg Xaa Gly
85 90 95

Phe Ile Xaa Ser Ile Cys Leu Ala Val Asn Pro Arg Ser Pro Ser Ala
100 105 110

Phe Trp Ala Cys Met Phe Ala Leu Ser Lys Ile Ala Glu Phe Gly Asp
115 120 125

Thr Met Phe Leu Val Leu Arg Lys Arg Pro Val Ile Phe Leu His Trp
130 135 140

Tyr His His Ala Val Val Leu Ile Leu Ser Trp His Ala Ala Ile Glu
145 150 155 160

Leu Thr Ala Pro Gly Arg Trp Phe Ile Phe Met Asn Tyr Leu Val His
165 170 175

Ser Ile Met Tyr Thr Tyr Tyr Ala Ile Thr Ser Ile Gly Tyr Arg Xaa
180 185 190

Pro Lys Ile Val Ser Met Thr Val Thr Phe Leu Gln Thr Leu Gln Met

```
        195                 200                 205

Leu Ile Gly Val Ser Ile Ser Cys Ile Val Leu Tyr Leu Lys Leu Asn
    210                 215                 220

Gly Glu Met Cys Gln Gln Ser Tyr Asp Asn Leu Ala Leu Ser Phe Gly
225                 230                 235                 240

Ile Tyr Ala Ser Phe Leu Val Leu Ser Ser Phe Phe Asn Asn Ala Tyr
                245                 250                 255

Leu Val Lys Lys Asp Lys Lys Pro Asp Val Lys Lys Asp
            260                 265
```